IL8 polymorphisms and overall survival in pazopanib- or sunitinib-treated patients with renal cell carcinoma

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Background: We evaluated germline single nucleotide polymorphisms (SNPs) for association with overall survival (OS) in pazopanib- or sunitinib-treated patients with advanced renal cell carcinoma (aRCC).

Methods: The discovery analysis tested 27 SNPs within 13 genes from a phase III pazopanib trial ($N = 241$, study 1). Suggestive associations were then pursued in two independent datasets: a phase III trial (COMPARZ) comparing pazopanib *vs* sunitinib ($N = 729$, study 2) and an observational study of sunitinib-treated patients ($N = 89$, study 3).

Results: In study 1, four SNPs showed nominally significant association ($P \leq 0.05$) with OS; two of these SNPs (rs1126647, rs4073) in *IL8* were associated ($P \leq 0.05$) with OS in study 2. Because rs1126647 and rs4073 were highly correlated, only rs1126647 was evaluated in study 3, which also showed association ($P \leq 0.05$). In the combined data, rs1126647 was associated with OS after conservative multiple-test adjustment ($P = 8.8 \times 10^{-5}$; variant *vs* reference allele hazard ratio 1.32, 95% confidence interval: 1.15–1.52), without evidence for heterogeneity of effects between studies or between pazopanib- and sunitinib-treated patients.

Conclusions: Variant alleles of *IL8* polymorphisms are associated with poorer survival outcomes in pazopanib- or sunitinib-treated patients with aRCC. These findings provide insight in aRCC prognosis and may advance our thinking in development of new therapies.

Renal cell carcinoma (RCC) is a heterogeneous collection of malignancies arising from the renal parenchyma, with $>200\,000$ new cases worldwide per year (Gupta *et al*, 2008; Escudier *et al*, 2013).

Although highly curable in its localised form by surgery, approximately a third of patients are diagnosed when metastatic spread has already occurred (Gupta *et al*, 2008); in addition,

approximately 30% of patients surgically treated for a localised primary tumour will eventually develop metastases over time (Janowitz *et al*, 2013). The development of targeted systemic treatment options has improved clinical outcomes in patients with advanced and metastatic RCC (Molina and Motzer, 2011; Escudier *et al*, 2013; Fisher *et al*, 2013). Initial treatment decisions are based on prognosis of the disease using risk assessment models. Such prognostic models, initially developed for stratification of patients with metastatic RCC undergoing cytokine treatment (Motzer *et al*, 1999), have recently been extended to apply to patients receiving targeted therapies (Heng *et al*, 2009, 2013).

Pazopanib and sunitinib are angiogenesis inhibitors with highest affinity for vascular endothelial growth factor receptors, platelet-derived growth factor receptors, and stem cell factor receptor c-Kit (Motzer *et al*, 2007; Sternberg *et al*, 2010), and are approved for the treatment of advanced RCC. Treatment guidelines (Motzer *et al*, 2014; Escudier *et al*, 2014b) include both therapies as first-line options for RCC. Progression-free survival (PFS) benefits were observed for both sunitinib (*vs* interferon-alfa) and pazopanib (*vs* placebo) in their respective pivotal phase III studies (Motzer *et al*, 2007; Sternberg *et al*, 2010), but overall survival (OS) benefits were either marginal or not statistically significant (Motzer *et al*, 2007; Sternberg *et al*, 2013). However, these OS analyses were confounded by crossover or access to other therapies after progression. A recently completed phase III randomised clinical trial (COMPARZ) comparing pazopanib *vs* sunitinib for RCC demonstrated similar efficacies but differential safety profiles for the two therapies (Motzer *et al*, 2013), and a randomised cross-over phase III study (PISCES) demonstrated a significant patient preference for pazopanib over sunitinib with health-related quality of life and safety as key influencing factors (Escudier *et al*, 2014a).

There is substantial heterogeneity between patients with advanced RCC in prognosis and in response to treatment with targeted therapies, including pazopanib and sunitinib, and biomarkers that are predictive of clinical benefit would facilitate evidence-based selection of particular agents or dosages for optimal treatment of individual patients. The most obvious candidate biomarkers in clear-cell metastatic RCC (e.g., von Hippel–Lindau status, hypoxia-inducible factor (HIF) expression) have not been proven to have predictive significance (Fisher *et al*, 2013). As noted in recent reviews (Funakoshi *et al*, 2014; Maroto and Rini, 2014), some retrospective and prospective studies have reported potential molecular prognostic or predictive factors, including serum biomarkers (Tran *et al*, 2012; Harmon *et al*, 2014), tumour biomarkers (Rini *et al*, 2010; Dornbusch *et al*, 2013), and germline genetic variants (Garcia-Donas *et al*, 2011; van der Veldt *et al*, 2011; Xu *et al*, 2011; Scartozzi *et al*, 2013). Specifically, genetic polymorphisms in genes involved in sunitinib pharmacokinetics (e.g., CYP3A5, NR1I3, and ABCB1) or mode of action (e.g., VEGFR3) have recently been reported to be associated with PFS or OS in advanced RCC (Garcia-Donas *et al*, 2011; Scartozzi *et al*, 2013). We have previously reported that genetic polymorphisms in *IL8* and *HIF1A* may be associated with PFS, and polymorphisms in *HIF1A*, *NR1I2*, and *VEGFA* may be associated with best response (Xu *et al*, 2011). None of these genetic studies reported any attempt to confirm the association(s) in an independent dataset. To our knowledge, no prognostic or predictive biomarkers have yet to be prospectively validated in multiple independent studies to reliably distinguish patients with advanced RCC who are likely to respond from those who will not. To identify genetic predictors for OS, the present study used three independent datasets totalling 1059 pazopanib- or sunitinib-treated patients with advanced RCC.

MATERIALS AND METHODS

Patients. The discovery study (hereafter study 1) used data from participants in trials NCT00334282/VEG105192 and NCT00387764/VEG107769. The preplanned confirmation study (hereafter study 2) used data from participants in the COMPARZ trial: NCT00720941/VEG108844 and NCT01147822/VEG113078. Studies 1 and 2 included patients who provided written informed consent both for the clinical study and for genetic research. These clinical studies were conducted in accordance with the Declaration of Helsinki; protocols and informed consent forms were reviewed and approved by Institutional Review Boards and Independent Ethics Committees according to local guidelines. *Post hoc*, additional confirmation was sought using an observational study (hereafter study 3); the protocol was approved by the medical ethics review board of each participating institution and each participant provided written informed consent (Garcia-Donas *et al*, 2011).

Patient characteristics have been described previously (Sternberg *et al*, 2010; Garcia-Donas *et al*, 2011; Xu *et al*, 2011; Motzer *et al*, 2013) (Table 1). Briefly, NCT00334282 was a randomised, double-blind, placebo-controlled, pivotal phase III pazopanib study for advanced and/or metastatic RCC (Sternberg *et al*, 2010). NCT00387764 was an open-label extension to NCT00334282 providing the option for placebo-treated patients who developed progressive disease to receive pazopanib; OS for these patients was calculated from the time of initiation of pazopanib treatment in NCT00387764. Of the 369 patients enrolled in NCT00334282 and NCT00387764 who received pazopanib, study 1 used data from 241 patients who provided consent and a blood sample for genetic research. COMPARZ was a phase III randomised clinical trial comparing pazopanib *vs* sunitinib for metastatic RCC (Motzer *et al*, 2013). Of 1110 patients enrolled in COMPARZ, study 2 used data from 729 patients who received either pazopanib ($N = 374$) or sunitinib ($N = 355$) and provided consent and a blood sample for genetic research. Study 3 was an observational study undertaken by the Spanish Oncology GenitoUrinary Group (SOGUG); sunitinib-treated patients with clear-cell advanced RCC were included ($N = 89$). Compared with the analysis reported previously by Garcia-Donas (27 patients died, median follow-up 21.2 months) (Garcia-Donas *et al*, 2011), this analysis uses data from extended follow-up for OS (50 patients died, median follow-up 36.9 months).

Procedures. For studies 1 and 2, germline DNA was extracted from peripheral blood (QiAamp DNA Blood Kit; Qiagen, Valencia, CA, USA). In the discovery analysis, 27 potential functional single nucleotide polymorphisms (SNPs) were selected from 13 candidate genes with evidence of involvement in angiogenesis or in the metabolism, disposition, or mode of action of pazopanib (Xu *et al*, 2011). Genotyping was conducted using single-base chain extension assays modified by GlaxoSmithKline (Research Triangle Park, NC, USA), TaqMan SNP assays (Applied Biosystems, Foster City, CA, USA), GoldenGate and Infinium genotyping assays (Illumina, San Diego, CA, USA), the KASPar SNP genotyping system (LGC Genomics, Hoddesdon, UK), and Sanger sequencing. For study 3, DNA was isolated from peripheral blood with FlexiGene DNA kit (Qiagen) or from saliva with Oragene DNA self-collection kits (DNA Genotek, Ottawa, Canada), and genotyping was conducted using the KASPar SNP genotyping system (Garcia-Donas *et al*, 2011).

All genotypes were called following the assay manufacturers' guidelines. Genotyping quality was confirmed by call rate, manual examination of cluster plots, concordance with previously reported allele frequencies, and checks of Hardy–Weinberg proportions

Table 1. Demographic and clinical characteristics of patients in the three studies

Characteristics	Discovery, Study 1 (pazopanib) N = 241	Confirmation, Study 2 (pazopanib or sunitinib) N = 729	Confirmation, Study 3 (sunitinib) N = 89
Age, median years (range)	60 (25–85)	61 (18–86)	65 (55–73)
Sex, n (%)			
Male	170 (71)	552 (76)	61 (69)
Race, self-reported, n (%)			
White	209 (87)	453 (62)	87 (98)
Non-white	32 (13)	276 (38)	2 (2)
Body mass index, median kg m^{-2} (range)	26 (14–46)	26 (15–55)	NA
ECOG PS or Karnofsky score, n (%)			
ECOG 0/KS 90–100	93 (39)	556 (76)	23 (28)
ECOG 1/KS 70–80	144 (60)	167 (23)	53 (64)
ECOG 2/KS ⩽60/missing[a]	4 (2)	6 (1)	13 (14)
MSKCC risk score, n (%)			
Favourable risk	94 (39)	211 (29)	27 (30)
Intermediate risk	130 (54)	420 (58)	44 (49)
Poor risk	2 (1)	73 (10)	2 (2)
Unknown	15 (6)	25 (3)	16 (18)
Time since initial diagnosis, n (%)			
⩽1 year	74 (31)	400 (55)	47 (53)
>1 year	139 (58)	329 (45)	42 (47)
Missing data	28 (12)	0	0
Prior nephrectomy status, n (%)			
Yes	216 (90)	611 (84)	76 (85)
No	23 (10)	118 (16)	13 (15)
Missing data	2 (<1)	0	0
LDH, n (%)			
⩽1·5 × ULN	215 (89)	673 (92)	82 (92)
>1·5 × ULN	16 (7)	45 (6)	4 (4)
Missing data	10 (4)	11 (2)	3 (3)
Haemoglobin, n (%)			
⩾LLN	129 (54)	445 (61)	55 (62)
<LLN	104 (43)	284 (39)	33 (37)
Missing data	8 (3)	0	1 (1)
Prior systemic treatment, n (%)			
Treatment-naive	128 (53)	729 (100)	89 (100)
Cytokine-pretreated	105 (44)	0	0
Missing data	8 (3)	0	0
Neutrophil count, n (%)			
⩽ULN	194 (81)	643 (88)	0
>ULN	39 (16)	82 (11)	0
Missing data	8 (3)	4 (1)	89 (100)
Platelet count, n (%)			
⩽ULN	184 (76)	629 (86)	0
>ULN	49 (20)	98 (13)	0
Missing data	8 (3)	2 (<1)	89 (100)
PFS, median weeks (95% CI)	38 (28–52)	48 (38–49)	55 (33–77)
OS, median months (95% CI)	25 (22–28)	32 (28–36)	27 (17–37)
Tumour objective response, n (%)[b]	83 (37)	241 (33)	39 (49)[c]

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; KS = Karnofsky score; LDH = lactate dehydrogenase; LLN = lower limit of normal range; MSKCC = Memorial Sloan-Kettering Cancer Center; OS = overall survival; PFS = progression-free survival; ULN = upper limit of normal range.
[a]One patient had missing baseline ECOG PS. All patients with ECOG PS of 2 or missing were from study VEG107769, had been randomised to the placebo arm of VEG105192, and later experienced disease progression while on treatment or during the follow-up period.
[b]Objective response represents complete response and partial response.
[c]Nine had missing data, 49% = 39 complete + partial responses/80 patients with data.

within self-reported non-Hispanic white patients and within self-reported East Asian patients.

Statistical analysis. In study 1 (discovery analysis), following Motzer *et al* (1999, 2002) and Heng *et al* (2009), baseline factors were first individually evaluated for association with OS using a univariate Cox proportional hazards model, and subsequently evaluated using multivariate stepwise model selection (forward selection at $P \leq 0.1$ to enter the model and backward selection at $P \leq 0.05$ to stay in the model) (Table 2). Each SNP was tested for association with OS using a multivariate Cox model, assuming an additive genetic model, and adjusting for the baseline factors identified by the stepwise model selection. SNPs showing nominal significance ($P \leq 0.05$ without adjustment for the number of SNPs tested) were considered for evaluation in study 2.

Table 2. Effect of baseline factors on overall survival in pazopanib-treated patients in the univariate and multivariate cox regression model in discovery study 1

Factors	Univariate		Multivariate[a]	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, increase/year	1.00 (0.98–1.01)	0.9	—	—
Sex, female vs male	1.24 (0.88–1.73)	0.2	—	—
Race, self-reported other vs white	0.92 (0.57–1.48)	0.7	—	—
BMI, per $kg\,m^{-2}$	0.95 (0.91–0.98)	0.002	0.95 (0.92–0.99)	0.008
MSKCC risk score, intermediate/poor vs favourable	1.93 (1.37–2.73)	0.0002	—	—
ECOG PS, 1 or 2 vs 0[b]	1.73 (1.25–2.42)	0.001	1.63 (1.12–2.38)	0.01
Haemoglobin, <LLN vs ≥LLN[b]	1.53 (1.12–2.10)	0.008	—	—
LDH, >1.5 × ULN vs ≤1.5 × ULN[b]	2.50 (1.41–4.42)	0.002	—	—
Prior nephrectomy status, no vs yes	1.33 (0.81–2.21)	0.3	—	—
Prior systemic treatment, treatment-naive vs cytokine-pretreated	1.24 (0.90–1.71)	0.2	—	—
Number of disease sites, ≥3 vs 1 or 2	1.24 (0.90–1.71)	0.003	1.56 (1.08–2.24)	0.02
Time from initial diagnosis to study entry, ≤1 year vs >1 year[b]	1.81 (1.29–2.54)	0.0006	1.50 (1.05–2.15)	0.03
Neutrophil count, ULN vs ≤ULN	1.82 (1.23–2.69)	0.003	1.66 (1.08–2.55)	0.02
Platelet count, >ULN vs ≤ULN	1.24 (0.86–1.80)	0.3	—	—
Study, VEG105192 vs VEG107769	1.06 (0.72–1.56)	0.8	—	—

Abbreviations: BMI = body mass index; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; LDH = lactate dehydrogenase; LLN = lower limit of normal range; MSKCC = Memorial Sloan-Kettering Cancer Center; ULN = upper limit of normal range.
[a]For the multivariate model, HR and P values were shown for the final set of stepwise selected variables only; these variables were included as covariate(s) in the analysis of the effect of each genetic marker.
[b]These factors are also included in the calculation of the MSKCC risk score (Motzer et al, 1999, 2002).

In studies 2 and 3 (preplanned and *post hoc* confirmation analyses, respectively), SNPs were tested for association with OS using a multivariate Cox model, assuming an additive genetic model. In study 2, analyses were adjusted for the same baseline covariates as in study 1, plus ancestry principal components to adjust for confounding by population structure (Price *et al*, 2006). In study 3, analyses were adjusted for baseline Memorial Sloan-Kettering Cancer Center (MSKCC) risk score and sex, as described previously (Garcia-Donas *et al*, 2011). In parallel, SNPs nominally significantly associated ($P \leq 0.05$) with either PFS or best response, as reported previously (Xu *et al*, 2011), were also tested for association with either PFS or best response in study 2. All analyses in study 2 were conducted separately in pazopanib-treated and in sunitinib-treated patients, and also in a combined analysis of all patients (with additional covariate adjustment for treatment). Two-tailed P values were reported, and patients with missing baseline covariates or missing genotypes were excluded on a per-analysis basis.

Estimates of the natural-log hazard ratio (HR) from the multivariate Cox model analyses for study 1, from the two treatment arms of study 2 and from study 3, were combined using inverse variance weighted meta-analysis, assuming fixed effects and an additive genetic model. Heterogeneity of effects was assessed using the I^2 index of heterogeneity and by Cochran's Q statistic. For meta-analysis, a conservative significance threshold was determined using Bonferroni correction for all discovery-only and discovery-plus-confirmation analyses that could have been conducted (27 SNPs × 4 sequential analyses with increasing cumulative sample sizes; threshold $0.05/27/4 = 4.6 \times 10^{-4}$). Statistical analyses were conducted using the R system (Free Software Foundation, Boston, MA, USA), version 3.0.1.

RESULTS

We analyzed data from 1059 patients with advanced/metastatic RCC: 241 pazopanib-treated patients in study 1 (discovery); 374 pazopanib-treated and 355 sunitinib-treated patients in study 2 (preplanned confirmation); 89 sunitinib-treated patients in study 3 (*post hoc* confirmation). There was some heterogeneity in demographic and baseline clinical characteristics across the three datasets (Table 1). Similarly, there was a modest difference between studies in OS (Table 1); this likely reflects differences in baseline characteristics, as there was no significant difference in OS between pazopanib- and sunitinib-treated patients within study 2 (Motzer *et al*, 2013).

Table 2 lists the baseline factors evaluated in study 1 (discovery). Poor/intermediate MSKCC risk score, poor Eastern Cooperative Oncology Group (ECOG) performance status, low haemoglobin, high lactate dehydrogenase, high neutrophil count, increased number of disease sites, shorter time since initial diagnosis, and low body mass index were significantly associated with poor OS in univariate analyses. Multivariate stepwise model selection identified ECOG status, neutrophil count, number of disease sites, time since initial diagnosis, and body mass index as significantly associated with OS ($P \leq 0.05$, Table 2). In analyses adjusted for these five baseline factors, five of the 27 SNPs studied were associated with OS at $P \leq 0.05$ in the discovery study 1 (Table 3). Although no single association was significant after adjusting for the 27 SNPs studied, the number of associations at $P \leq 0.05$ was higher than expected by chance, and therefore we hypothesised that some of these associations might be confirmed in a larger independent dataset. Excluding the SNP where only two patients carried the variant genotype, four SNPs were analyzed further.

We hypothesised that in patients with advanced RCC, genetic effects on OS could be similar for angiogenesis inhibitors in the same class (i.e., pazopanib and sunitinib). Therefore, for follow-up of the discovery findings, we tested the four SNPs in both the pazopanib and sunitinib arms of study 2. In analysis of all patients in study 2, adjusted for treatment received, two *IL8* SNPs were associated with OS at $P \leq 0.05$ (rs1126647, Figure 1A; rs4073, Supplementary Figure S1). Genotypes at these two *IL8* SNPs were strongly correlated with each other (study 1 $r^2 = 0.79$; $P < 0.0001$),

Table 3. Association between genetic markers and overall survival in pazopanib-treated patients in discovery study 1

Gene	Polymorphism	rs Number	Minor Allele Frequency, %	P value[a]	HR[a] (95% CI)
CYP3A4	− 392A > G	rs2740574	G 4.8	0.5	0.78 (0.39–1.55)
CYP3A5	6986A > G	rs776746	A 11.8	0.98	1.00 (0.68–1.45)
NR1I2	− 25385C > T	rs3814055	T 39.0	0.02	1.34 (1.04–1.73)
NR1I2	7635A > G	rs6785049	G 40.6	0.7	0.96 (0.74–1.24)
NR1I2	10620C > T	rs1054190	T 9.0	0.7	1.11 (0.71–1.73)
ABCB1	1236C > T	rs1128503	T 43.2	0.054	1.25 (1.00–1.58)
ABCB1	2677G > T/A (A893S/T)	rs2032582	T/A 45.4	0.3	1.14 (0.89–1.47)
ABCB1	3435C > T	rs1045642	T 47.7	0.1	1.22 (0.94–1.58)
ABCG2	34G > A (V12M)	rs2231137	A 9.0	0.5	0.85 (0.55–1.33)
ABCG2	421C > A (Q141K)	rs2231142	A 13.5	0.7	1.06 (0.74–1.53)
ABCG2	869C > T (Q126X)	rs72552713	T 0.4	0.03[b]	4.81 (1.15–20.18)
VEGFA	− 2578A > C	rs699947	C 47.9	0.2	0.85 (0.66–1.09)
VEGFA	− 1498C > T	rs833061	T 47.7	0.2	0.85 (0.66–1.09)
VEGFA	− 1154G > A	rs1570360	A 33.5	0.09	1.25 (0.96–1.63)
VEGFA	− 634G > C	rs2010963	C 27.8	0.2	0.83 (0.62–1.11)
VEGFA	936C > T	rs3025039	T 15.3	0.4	1.15 (0.82–1.62)
VEGFR2	− 604T > C	rs2071559	C 43.5	0.4	0.90 (0.70–1.14)
VEGFR2	889G > A (V297I)	rs2305948	A 8.9	0.4	1.20 (0.77–1.86)
VEGFR2	1416A > T (Q472H)	rs1870377	T 22.6	0.3	0.86 (0.64–1.16)
VEGFR3	1480A > G (T494A)	rs307826	G 8.6	0.2	1.36 (0.85–2.19)
PDGFRα	− 573G > T	rs1800812	T 21.7	0.3	0.86 (0.66–1.13)
IL8	2767A > T	rs1126647	T 42.8	0.007	1.45 (1.11–1.91)
IL8	− 251T > A	rs4073	A 49.8	0.02	1.36 (1.04–1.76)
FGF2	224C > T	rs1449683	T 8.8	0.3	1.29 (0.78–2.12)
FGFR2	IVS2 + 906C > T	rs2981582	T 38.8	0.008	1.40 (1.09–1.81)
HIF1α	1772C > T (P582S)	rs11549465	T 10.3	0.6	0.88 (0.58–1.34)
HIF1α	1790G > A (A588T)	rs11549467	A 3.2	0.6	0.84 (0.42–1.65)

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]The HR and P values were from additive genetic models. The P values were nominal values without adjustment for the number of SNPs tested; HR values represent the per variant allele allelic HR.
[b]Although the ABCG2 rs72552713 was nominally significantly associated with overall survival, this association was driven by the only two patients with the variant CT genotype, and thus was not further discussed in this manuscript.

with rs1126647 (IL8 2767A > T) showing stronger association in both study 1 and study 2. Therefore, we focused on this SNP for a detailed description of the results. Given the inclusion of sunitinib-treated patients in study 2 but not in study 1, and a lack of other available genetic data for pazopanib-treated patients with advanced RCC, we sought additional confirmation for the association of rs1126647 in study 3.

In study 1 (pazopanib-treated, $N = 241$), rs1126647 was significantly associated with OS ($N = 186$; 125 events; $P = 0.007$, per allele HR = 1.45, 95% confidence interval (CI): 1.11–1.91; 18 patients had missing genotype data and 37 patients had missing data for baseline factors) (Table 3, Figure 2). The effect size estimate was similar when baseline factors were not adjusted for ($N = 223$; 148 events; $P = 0.003$, per allele HR = 1.44, 95% CI: 1.13–1.83) (Figure 1A).

In study 2 (pazopanib- or sunitinib-treated, $N = 729$), rs1126647 was significantly associated with OS ($N = 690$; 287 events; $P = 0.018$, HR = 1.23, 95% CI: 1.04–1.46, with adjustment for treatment; 10 patients had missing genotype data and 29 patients had missing data for baseline factors). The effect size estimate was similar when baseline factors were not adjusted for ($N = 719$; 299 events; $P = 0.014$, HR = 1.24, 95% CI: 1.04–1.46) (Figure 1B). The HRs for association between rs1126647 and OS were not significantly different between pazopanib-treated patients

($N = 353$; 146 events; $P = 0.53$, HR = 1.08, 95% CI: 0.84–1.40) and sunitinib-treated patients ($N = 337$; 141 events; $P = 0.008$, HR = 1.39, 95% CI: 1.09–1.77) in study 2 (Figure 2), with overlapping CIs (Figure 3) and no significant genotype by treatment interaction effect ($P = 0.23$). The lack of a nominally significant association in pazopanib-treated patients in study 2 ($P = 0.53$) precludes straightforward interpretation of these results, which is an issue we discuss further.

In study 3 (sunitinib-treated, $N = 89$), a significant association between rs1126647 and OS, with similar effect size, was observed ($N = 88$; 50 events; $P = 0.034$, HR = 1.62, 95% CI: 1.04–2.54; one patient had missing genotype data) (Figures 1C and 2).

Meta-analysis of results from all three studies showed overall a significant association between IL8 rs1126647 genotype and OS ($P = 8.8 \times 10^{-5}$, HR = 1.32 per T allele, 95% CI: 1.15–1.52) (Figure 2) that was significant after Bonferroni correction for all discovery-only and discovery-plus-confirmation analyses that could have been conducted using the available data (threshold $P \leq 4.6 \times 10^{-4}$). There was no significant heterogeneity in genetic effect size between studies ($I^2 = 19\%$, Cochran's Q = 3.73, 3 degrees of freedom, $P = 0.29$).

Our previous pharmacogenetic analyses of pazopanib clinical trials for RCC (using data from study 1 plus an additional clinical trial that did not have OS data) suggested that three SNPs in the
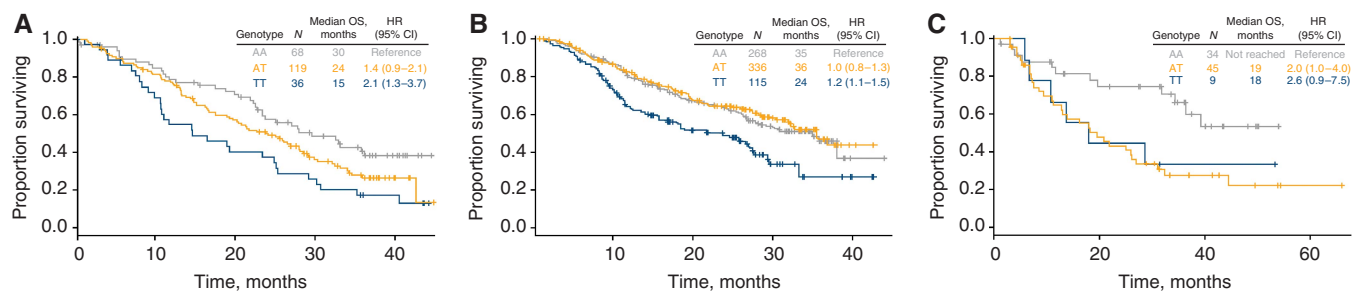
Figure 1. Overall survival Kaplan–Meier curves for patients by each *IL8* 2767A>T (rs1126647) genotype. (**A**) Pazopanib-treated patients in discovery study 1 (from NCT00334282 and NCT00387764): of the 241 patients, 223 had *IL8* genotype data and were included in this plot (including the 37 patients who had missing data for baseline factors). The remaining 18 patients had missing genotype data. (**B**) Pazopanib- or sunitinib-treated patients in confirmation study 2 (from COMPARZ): of the 729 patients, 719 had *IL8* genotype data and were included in this plot (including the 29 patients who had missing data for baseline factors). Ten patients had missing genotype data. (**C**) Sunitinib-treated patients in confirmation study 3 (SOGUG study): 88 of the 89 patients had *IL8* genotype data and were included in this plot; one patient had missing genotype data. The curves show the proportion of patients in each genotype group who survived (*y* axis) *vs* time in months (*x* axis). Vertical bars on the survival curves indicate censored observations. The HR was adjusted for covariates comparing each of the variant genotype (AT or TT) with the reference genotype (AA). AA, reference genotype; AT, variant heterozygote genotype; TT, variant homozygote genotype.
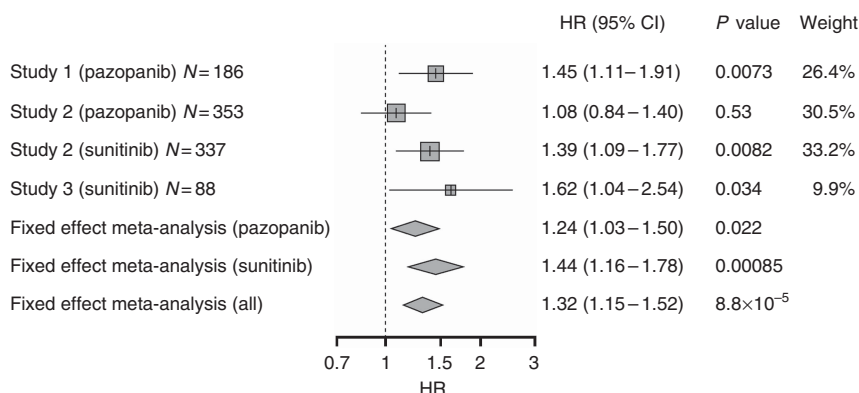

Figure 2. Forest plot of meta-analysis association results between *IL8* rs1126647 polymorphism and OS across three independent studies (with confirmation study 2 split into pazopanib- and sunitinib-treated subgroups). The HR was per variant T allele compared with reference A allele using an additive genetic model.

IL8 and *HIF1A* genes may be associated with PFS, and that five SNPs in the *HIF1A, NR1I2,* and *VEGFA* genes may be associated with best response (Xu *et al*, 2011). None of these SNPs showed nominally significant association (at $P \leq 0.05$) with either PFS or best response in follow-up analyses in the subset of pazopanib-treated patients from study 2, but *IL8* SNPs were weakly associated with PFS in sunitinib-treated patients.

DISCUSSION

Several antiangiogenesis agents are available for the treatment of advanced/metastatic RCC. However, few reliable predictors for treatment outcomes are available. The only externally validated models are the MSKCC or the International Metastatic Renal-Cell Carcinoma Database Consortium (IMDC) criteria that include baseline clinical factors to separate patients into risk categories with different prognoses (Motzer *et al*, 2002; Heng *et al*, 2013). Therefore, there is a growing interest in the field to explore pre-treatment demographic and clinical factors, serum/tissue biomarkers, and germline genetic markers that are potentially associated with efficacy endpoints. Here, using data from 1059 patients in three independent datasets, we report that rs1126647 in *IL8* is associated with OS in pazopanib- or sunitinib-treated patients with advanced RCC. Although the variant *IL8* genotype (TT) was

associated with shorter OS than other genotypes, all genotype subgroups had survival benefit from treatment with pazopanib or sunitinib. For example, the median OS was 21.4–23.7 months for patients with the variant TT genotype and 27.8–35.5 months for the other genotypes in COMPARZ (Figure 3), all of which were substantially improved compared with historical survival data in advanced RCC when cytokines were the mainstay treatment (median survival 13 months) (Motzer *et al*, 2000). As the magnitudes of OS benefit vary depending on *IL8* genotypes, alternative sequencing or combination treatment strategies that are based on genotyping could be explored in the future as new therapies become available. Furthermore, the *IL8* genotype data could be incorporated into a prognostic model such as the existing IMDC model to improve the predictions of patients' clinical outcomes (Heng *et al*, 2013).

Progression-free survival has been used as a primary endpoint in some oncology clinical trials and has been considered an acceptable surrogate for OS in some settings (Shea *et al*, 2013; Bria *et al*, 2015). We were able to demonstrate association of *IL8* rs1126647 with OS but not with PFS. However, surrogacy with respect to the effect of a targeted therapy need not imply surrogacy with respect to effects of genetic differences, which may have distinct mechanism(s) of action (Fleming and DeMets, 1996). This raises the possibility that *IL8* variants may be associated with OS irrespective of treatment.
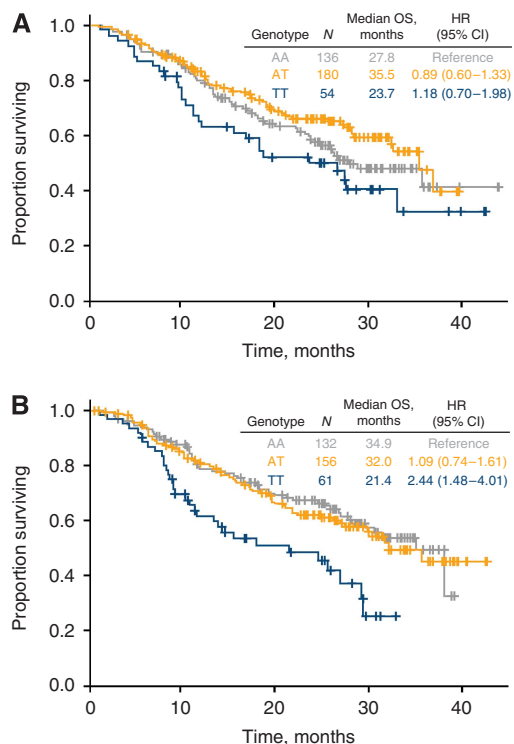
Figure 3. Overall survival (OS) Kaplan–Meier curves for *IL8* 2767A>T (rs1126647) genotype in confirmation study 2 (from COMPARZ) for (**A**) pazopanib-treated patients and (**B**) sunitinib-treated patients. Of the 729 patients, 719 had *IL8* genotype data and were included in this plot (including the 29 patients who had missing data for baseline factors). Ten patients had missing genotype data. AA, reference genotype; AT, variant heterozygote genotype; TT, variant homozygote genotype.

As with many cancer therapies, the benefit of antiangiogenic therapy is often transient in the metastatic disease setting, and there has been an ongoing search to identify mechanisms of resistance. Although clinical examples of clearly established mechanisms of resistance to angiogenesis inhibitors remain limited, findings from cell culture and murine model studies have revealed that activation of alternate or redundant signalling pathways may represent one such mechanism (Mizukami *et al*, 2005; Huang *et al*, 2010). The IL8 protein possesses mitogenic and angiogenic properties (Koch *et al*, 1992), and IL8-mediated angiogenesis was identified as a key compensatory mechanism of resistance to sunitinib in murine models of RCC (Huang *et al*, 2010). The *IL8* variant alleles evaluated in this study have been previously shown to be associated with increased gene expression (Hacking *et al*, 2004). Overexpression of IL8 is correlated with tumour stage, disease progression, and recurrence in various cancers (Yuan *et al*, 2005), as well as worse prognosis in localised RCC (Rini *et al*, 2010). In patients receiving pazopanib or sunitinib, high baseline serum IL8 levels were associated with shorter PFS and/or OS, suggesting that serum IL8 concentrations may be a prognostic or predictive factor for metastatic RCC (Liu *et al*, 2011; Tran *et al*, 2012; Harmon *et al*, 2014). One could therefore speculate that patients carrying the high-expression *IL8* variants may have more aggressive tumours and thus reduced survival *vs* those carrying the low-expression genotypes. It may be reasonable to consider IL8 blockade as a potential therapeutic target in future drug development for this patient subset.

Pharmacogenetic studies are often hampered by small sample sizes and limited availability of validation studies with eligibility criteria and treatment regimen similar to the discovery study. Strengths of the present study include a hypothesis-driven approach in a relatively large sample size study, the availability of one discovery and two confirmatory datasets, and detailed data on patient baseline characteristics. The prospective collection of germline DNA samples during pazopanib clinical trials enabled the evaluation of the effects of genetic markers on clinical response. Our evidence for association between OS and *IL8* rs1126647 in pazopanib- and sunitinib-treated patients with RCC is based on a combined analysis of all data available for this study. Clearly, these data were accumulated in stages, and the inclusion of study 3 data in our analysis was *post hoc*. Nonetheless, the strength of association based on all available data ($P = 8.8 \times 10^{-5}$) remains significant after a conservative multiple testing correction that accounts for the number of stages of data accumulation and also for the total number of SNPs that could have been followed through these stages (threshold $P \leq 4.6 \times 10^{-4}$). The evidence for association of *IL8* rs1126647 in patients treated with either pazopanib or sunitinib is supported by substantially overlapping 95% CIs from treatment-specific meta-analyses (pazopanib 95% CI: 1.03–1.50, sunitinib 95% CI: 1.16–1.78) (Figure 2). However, the association between OS and *IL8* rs1126647 in patients with RCC requires *bona fide* prospective validation in further independent studies.

In conclusion, data from the present study suggest that variant alleles (associated with high expression) in the *IL8* gene are associated with poorer survival outcome in patients with RCC who have received pazopanib or sunitinib. These findings provide additional scientific insight in the prognosis of advanced RCC after antiangiogenesis therapy, and may advance our thinking in developing new therapies.

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CONFLICT OF INTEREST

CFX, TJ, ZX, LM, JCW, CFS, and LNP are employees and stockholders of GlaxoSmithKline. NB and KCD were GlaxoSmithKline employees at the time of this study. JGD has received honoraria from GlaxoSmithKline, Pfizer, Novartis, and Bayer. CNS has received honoraria from GlaxoSmithKline, Pfizer, Novartis, and Bayer. IDD has been an advisory board member or chair for GlaxoSmithKline, Pfizer, Novartis, Janssen, Medivation, Sanofi, Bayer, Astellas, Ipsen, and Bristol-Myers Squibb, but all honoraria were directly donated to ANZUP Cancer Trials Group. TKC has received consultant compensation from GlaxoSmithKline, Pfizer, AVEO, Novartis, Genentech, Bayer, and Onyx. EE has been an advisory board member for GlaxoSmithKline, Pfizer, Novartis, and Bayer, but all honoraria were directly donated to UNDESO (foundation for the development of oncology research). RJM reports grants to his institution from GlaxoSmithKline, Pfizer, Novartis, and AVEO Oncology; he has received compensation from GlaxoSmithKline for travel and consultant compensation

from Pfizer and Genentech. CR-A declares that she has no conflicts of interest.

AUTHORS CONTRIBUTIONS

CFX, JG-D, ZX, and CR-A contributed to study design and conduct, acquisition of data, and data interpretation. TJ and NB contributed to study design, statistical analysis, and data interpretation. TKC, CNS, IDD, and RJM contributed to study design, acquisition of clinical data and DNA samples, and data interpretation. KCD, LM, and LNP contributed to study design and data interpretation. EE contributed to acquisition of data and data interpretation. JCW and CFS contributed to data interpretation. All authors contributed to the writing of this report and approved the final version.

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