Clear-cell Renal Cell Carcinoma: Molecular Characterization of IMDC Risk Groups and Sarcomatoid Tumors

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Abstract

In advanced clear-cell renal cell carcinoma, the Checkmate214 study showed superior outcomes with ipilimumab-nivolumab in International Metastatic RCC Database Consortium intermediate-/poor-risk patients and with sunitinib in good-risk patients. We demonstrate how underlying molecular subtypes and angiogenic gene expression can explain these differences and can characterize the heterogeneous International Metastatic RCC Database Consortium intermediate-risk group.

Introduction: Recent trials have suggested predictive biomarkers in advanced clear-cell renal cell carcinoma (accRCC): International Metastatic RCC Database Consortium (IMDC) good risk or angiogenic gene signature for sunitinib and IMDC intermediate/poor risk for ipilimumab-nivolumab and T-effector cell signature or sarcomatoid dedifferentiation for atezolizumab-bevacizumab. We hypothesized that earlier described molecular subtypes, ccrc1 to ccrc4, could provide similar information as a single generic biomarker and molecularly characterize the heterogeneous intermediate-risk group. **Patients and Methods:** Patients with accRCC treated with systemic therapies were included. We assessed associations between the 5 biomarkers and their impact on progression-free survival (PFS) and response rate (RR) on first-line sunitinib or pazopanib. The cutoff percentage of sarcomatoid dedifferentiation with optimal discriminative value was determined. **Results:** In total, 430 patients were included (163 with molecular data). The molecular ccrc2 subtype identified tumors with higher angiogenic gene expression across IMDC risk groups: prevalence was high in IMDC good risk and low in IMDC poor risk ($P < .001$). Molecular subtype, IMDC, and angiogenic gene expression had comparable C-indices to predict PFS and RR (range, 60%-66%). The ccrc2 subtype and angiogenic gene expression were positive predictors of PFS in IMDC intermediate-risk patients ($P = .006$; $P = .04$). Immune signature did not differ between IMDC groups, but was strongly correlated with molecular subtype ($P = .8$ and $P = .0007$). A cutoff value of 25% sarcomatoid differentiation discriminated tumors with distinct molecular characteristics and therapeutic sensitivity. **Conclusion:** In accRCC, molecular subtypes can explain differences in IMDC risk group, expression of angiogenesis and immune response genes, and sarcomatoid dedifferentiation. They

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can identify molecularly different patient populations within the heterogeneous IMDC intermediate group and select patients for systemic therapies.

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Introduction

Clear-cell renal cell carcinomas (ccRCCs) are hallmarked by hypervascularity on the one hand and their capacity to elicit an immune response on the other hand.¹ Advanced disease (accRCC) may therefore be treated with angiogenesis inhibitors, immune checkpoint inhibitors (ICPIs), or a combination strategy.²⁻⁷ Outcomes on different therapies are, however, highly variable, and in the absence of predictive biomarkers, the optimal way of patient selection is unclear.

Only very recently have some advances in the field of predictive biomarkers been made. The phase III Checkmate214 trial (NCT0221749) compared ipilimumab-nivolumab with the angiogenesis inhibitor sunitinib in first-line therapy, after stratification for International Metastatic RCC Database Consortium (IMDC) risk group.^{7,8} Ipilimumab-nivolumab showed efficacy across all IMDC risk groups and was superior over sunitinib in intermediate-/poor-risk patients. It is therefore recommended in this population. Sunitinib, on the other hand, yielded favorable outcomes in good-risk patients, and sunitinib or pazopanib are recommended for these patients.² However, the IMDC risk group has important limitations as a biomarker. It is based on clinical risk factors that

may change over time, and the majority of patients are intermediate-risk, a group that is known to be very heterogeneous in terms of prognosis and treatment outcome.⁸⁻¹⁰

The phase III IMmotion151 trial (NCT02420821) compared atezolizumab-bevacizumab with sunitinib in first-line treatment and validated predictive gene signatures.^{4,11} A T-effector cell (T_{eff}) signature and programmed death ligand 1 (PD-L1) expression were associated with favorable outcomes on atezolizumab-bevacizumab and were similar across risk groups. An angiogenic gene signature was associated with favorable outcomes on sunitinib, and was higher in good-risk patients. Patients with sarcomatoid tumors responded poorly to sunitinib and had lower angiogenic signatures, but higher T_{eff}-signatures and PD-L1 expression. Yet, although these signatures are very promising, they have been developed specifically for sunitinib and atezolizumab-bevacizumab. Therefore, they only partly reflect intrinsic tumor biology and cannot be extrapolated to other treatments.

Another predictive marker that has been described in accRCC are 4 molecular subtypes, ccrc1 to ccrc4.¹² These subtypes were established based on unsupervised clustering of whole genome mRNA-expression data, and therefore reflect intrinsic differences in

Table 1 Correlation of Different Biomarkers With Outcome on First-line Sunitinib or Pazopanib

	N	PFS, mos	P	C-index (95% CI)	RR, %	P	C-index (95% CI)	OS, mos	P
IMDC	145								
Good	21	25	.0002	0.60 (0.55-0.65)	63	.01	0.62 (0.54-0.70)	72	<.0001
Intermediate	95	13			46			24	
Poor	29	5			19			12	
ccrc1-4 subtype	145								
ccrc1	42	12	<.0001	0.60 (0.57-0.68)	34	.01	0.66 (0.57-0.75)	23	.0002
ccrc2	63	19			57			31	
ccrc3	9	10			63			50	
ccrc4	31	5			25			11	
Angio score	145								
High	74	19	<.0001	0.64 (0.58-0.69)	56	.01	0.60 (0.50-0.70)	28	.005
Low	71	8			33			19	
Sarcomatoid	366								
<25%	344	11	<.0001		46	.001		24	<.0001
≥25%	22	3			10			8	

The discriminative power of predictors of RR and PFS was quantified with Harrells C-index (Harrell et al,¹⁸ JAMA 1982). Favorable IMDC risk group, ccrc2 or ccrc3 molecular subtype, High Angio score, and < 25% sarcomatoid dedifferentiation are predictors of favorable outcome. Abbreviations: CI = Confidence interval; IMDC = International Metastatic RCC Database Consortium; N = number; OS = overall survival; PFS = median progression-free survival; RR = response rate.

Figure 1 Impact on PFS and Mutual Correlations Between IMDC Risk Group, ccrc1 to ccrc4 Molecular Subtype, Angio Score, and Immune Score. A-C, ccrc2 or ccrc3 Subtype, High Angio Score, and More Favorable IMDC Risk Group Were Correlated With Longer PFS on First-line Sunitinib or Pazopanib. D, E, Within the IMDC Intermediate Risk Group, Molecular Subtype and Angio Score Were Predictive for PFS. F, The IMDC Good Risk Group Was Enriched for the ccrc2 Subtype, and the IMDC Poor Risk Group Was Enriched for the ccrc4 Subtype. G-I, The ccrc2 Subtype Identified Tumors With Higher Angio Scores Across IMDC Subgroups. J-M, Immune Score did Not Differ Between IMDC Risk Groups, but was Different Between the ccrc1 to ccrc4 Molecular Subtypes. The ccrc4 Subtype and a Subgroup of the ccrc2 Subtype had High Immune Scores. The Median Angio and Immune Scores are Reported in Graphs G, H, J, and K. The Expression of Individual Angiogenesis and Immune Genes According to IMDC Risk Group and Molecular Subtype Is Shown in Supplemental Tables 2 and 3 (in the Online Version)

Abbreviations: IMDC = International Metastatic RCC Database Consortium; PFS = progression-free survival.

tumor biology. They also differ in terms of immune infiltrate, cytogenetic anomalies, sarcomatoid dedifferentiation, methylation, and mutation profile in the tumor. The subtypes are prognostic for overall survival (OS) after nephrectomy and after metastasectomy with curative intent.^{12,13} The prognostic value of similar subtypes has been described by others in the setting of nephrectomy with curative intent.^{14,15} However, the ccrc1 to ccrc4 subtypes are also predictive for outcome on first-line sunitinib or pazopanib.^{12,16} ccrc2 tumors are characterized by pro-angiogenic gene expression and ccrc3 tumors by a normal-kidney-like gene expression pattern.^{12,17} Both ccrc2 and ccrc3 have a good prognosis and favorable outcomes with sunitinib or pazopanib. A subset of ccrc2

tumors also exhibit an active immune response. The 2 other subtypes, ccrc1 and ccrc4, are marked by upregulation of MYC-targets. ccrc1 tumors have an intermediate prognosis and outcome on sunitinib or pazopanib and an immune cold phenotype, with little T_H1-cell infiltration. On the other hand, ccrc4 tumors have a very poor prognosis and outcome on sunitinib or pazopanib, but are marked by a strongly inflamed, yet exhausted immune phenotype and are often sarcomatoid.

We hypothesized that the ccrc1 to ccrc4 molecular subtypes can explain differences in IMDC risk group, angiogenic signature, T_H1-signature, and sarcomatoid dedifferentiation, thus providing a maximum of information as a single biomarker. Therefore, the

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Figure 1 Continued

major aims of this study were to assess molecular differences between tumors in function of IMDC risk group and sarcomatoid dedifferentiation and correlate these with outcome on first-line sunitinib or pazopanib. A secondary aim was to characterize the heterogeneous IMDC intermediate-risk group on a molecular level.

Patients and Methods

Patients and Tissue

The CIT-rein database was searched for patients of whom the clinical and molecular data addressed in this study were available. The CIT-rein database is a French-Belgian multicenter database that includes patients with accRCC treated with systemic therapy or metastasectomy, containing over 600 cases. Patients are included after informed consent, and both clinical data and tumoral tissue are collected in a prospective way. Primary tumor tissue is collected at time of nephrectomy, before the start of any systemic therapy. Clear-cell histology and sarcomatoid dedifferentiation are confirmed by an expert uro-pathologist (N.R.L., E.L., V.V.). Outcome is systematically measured with Response Evaluation Criteria In Solid Tumors (RECIST) by one of the authors (B.B.). Drugs are dosed according to their manufacturer's label, with dose reductions as needed. Timing of assessments is at the discretion of the treating physician, usually once every 3 months. The study was approved by the ethics committees of the partaking centers.

Molecular Profiling

In patients from CIT-rein from whom fresh frozen primary tumor is available, expression of 98 genes is routinely determined with a custom panel (Fluidigm). This panel contains, among others, genes involved in angiogenesis and immune response. Molecular subtype is determined with an established 35-gene classifier algorithm as described previously.¹²

Angiogenic gene expression was quantified using 9 available genes involved in vascular endothelial growth factor (VEGF)-dependent angiogenesis. Fold changes were summarized as one "Angio score," using principal component analysis. Samples were also labeled as "Angio high" or "Angio low," depending on whether their Angio score was above or equal/below the median. Similarly, an "Immune score" was calculated using 13 available genes involved in immune response. As all genes are part of a routine panel, these scores were not trained as predictors for treatment outcome, nor is the Immune score an optimal signature of T_{eff}-cells.

Statistical Analysis

The optimal cutoff value for sarcomatoid dedifferentiation in order to predict progression-free survival (PFS) and overall survival (OS) was determined after considering all possible cutoffs in a Cox proportional hazard model, and selecting the dichomatization leading to the highest likelihood.

Figure 2 Sarcomatoid ccRCC. A, B, A Percentage of 25% Sarcomatoid Dedifferentiation Had the Optimal Discriminative Power to Predict Outcome On First-line Sunitinib or Pazopanib. C-F, ccRCC With $\geq 25\%$ Sarcomatoid Dedifferentiation Had Very Poor Outcomes on First-line Sunitinib or Pazopanib, Compared With 0% to 24%. In a Small Case Series, Outcomes on ICPIs Were Promising. Survival Curves of Patients With 0% or 1% to 24% Sarcomatoid Dedifferentiation Were Overlapping. G-J, ccRCC With $\geq 25\%$ Sarcomatoid Dedifferentiation Were Molecularly Different From ccRCC With 0% to 24% Sarcomatoid Dedifferentiation. They Were Mostly IMDC Poor Risk, ccrc4 Molecular Subtype, and Angio Low. The Median Angio and Immune Scores are Reported in Graphs I and J

Establishing 25% as the optimal cut-off percentage for sarcomatoid dedifferentiation

Outcome of 366 ccRCC with 0%, 1-24% or $\geq 25\%$ sarcomatoid dedifferentiation on first-line sunitinib or pazopanib, and of 92 patients on immune checkpoint inhibitors (4 with $\geq 25\%$ sarcomatoid dedifferentiation)

Abbreviations: ccRCC = clear-cell renal cell carcinoma; ICPIs = immune checkpoint Inhibitors; IMDC = International RCC Database Consortium; OS = overall survival; PFS = progression-free survival.

To assess the predictive impact of different biomarkers, we focused on their impact on response rate (RR) and PFS, as OS is heavily influenced by prognostic factors and subsequent therapies. The association of predictors with RR was analyzed using logistic regression. Their association with PFS was analyzed with Cox proportional hazards regression.

The Kruskal-Wallis and Mann-Whitney *U* tests were used to compare expression of individual genes between IMDC groups, molecular subtypes, and sarcomatoid yes/no. The associations between IMDC risk groups, molecular subtype, Angio high/low, Immune high/low, and sarcomatoid yes/no were tested with the Fisher exact test.

Analyses were done using SAS 9.4, GraphPad Prism 7.2, and XLStat 2018.7.

Results

Molecular Differences Between IMDC Risk Groups

Molecular data and IMDC risk group were available for 163 patients, 145 of whom were treated with first-line sunitinib or pazopanib (see [Supplemental Table 1](#) in the online version).

A more favorable IMDC risk group, a ccrc2 or ccrc3 molecular subtype, and higher Angio score were all positive predictors for longer PFS and higher RR on first-line sunitinib or pazopanib ([Table 1](#); [Figure 1](#)). The discriminative value of these predictors,

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Figure 2 continued

quantified by their C-index, was comparable. The combination of markers did not result in a meaningful increase in discriminative value.

IMDC risk group, molecular subtype, and Angio score were strongly associated with each other (Figure 1). The pro-angiogenic ccrcc2 molecular subtype could identify patients with higher Angio scores across IMDC risk groups. The IMDC good-risk group was enriched for the ccrcc2 subtype and Angio-high tumors (both 77%; $P < .001$). The IMDC poor-risk group was enriched for the unfavorable ccrcc4 subtype (41%) and Angio-low tumors (74%). The IMDC intermediate-risk group was heterogeneous, with a normal distribution of the molecular subtypes (46% ccrcc2) and 50% Angio-high and -low tumors. The expression of specific targets of sunitinib and pazopanib (VEGFR1, -2, -3) was highest in IMDC good-risk patients and lowest in IMDC poor-risk patients (see Supplemental Table 2 in the online version). Within the intermediate-risk group, the ccrcc2 subtype could identify tumors with higher angiogenic gene expression. Indeed, upon bivariable analysis, the differences in Angio score between IMDC risk groups became non-significant after correction for molecular subtype. Within the IMDC intermediate-risk group, both molecular subtype and Angio score were predictive for PFS.

The expression of genes related to immune response, summarized by the Immune score, did not differ between IMDC risk groups

($P = .7$). Contrarily, the differences in the Immune score between molecular subtypes were highly significant ($P = .0007$). ccrcc4 tumors, and a subgroup of ccrcc2 tumors, were marked by high Immune scores (Figure 1; Supplemental Table 3 in the online version). Thus, molecular subtypes could identify tumors with high immune gene expression across IMDC risk groups.

Sarcomatoid ccRCC

The impact of sarcomatoid dedifferentiation was tested in 366 patients treated with first-line sunitinib or pazopanib (see Supplemental Table 4 in the online version). The optimal cutoff associated with PFS and OS was $\geq 25\%$ sarcomatoid dedifferentiation. PFS and OS curves for patients with 0% or 1% to 24% sarcomatoid dedifferentiation were overlapping (Figure 2). On multivariable analysis, sarcomatoid dedifferentiation $\geq 25\%$ was independently associated with PFS (hazard ratio, 2.9; 95% confidence interval, 1.8–4.5) and OS (hazard ratio, 2.3; 95% confidence interval, 1.4–3.7) (see Supplemental Tables 5 and 6 in the online version). For tumors with 0% to 24% versus $\geq 25\%$ sarcomatoid dedifferentiation, RR was 46% versus 10% ($P = .001$), and PFS was 11 months versus 3 months ($P < .0001$) (Table 1).

Molecular differences and IMDC risk group were tested in 163 patients. Compared with tumors with 0% to 24% sarcomatoid dedifferentiation, tumors with $\geq 25\%$ dedifferentiation were

typically IMDC poor risk (62%; $P = .0004$) and ccrcc4 subtype (62%; $P = .0001$). They had lower Angio scores ($P = .009$) but no difference in Immune scores ($P = .35$). Tumors with 0% versus 1% to 24% sarcomatoid dedifferentiation had similar IMDC risk groups and Angio scores (see [Supplemental Tables 7 and 8](#) in the online version).

In an exploratory analysis, we assessed outcomes of 92 patients treated with ICPI, mostly in the second- or a consecutive line. The 4 tumors with $\geq 25\%$ sarcomatoid dedifferentiation all responded, whereas RR in 88 others was only 16%. PFS curves for 0% and 1% to 24% sarcomatoid dedifferentiation were overlapping and inferior to those for $\geq 25\%$.

Pooling of IMDC Intermediate and Poor Risk Groups

As phase III trials have typically reported outcomes of pooled IMDC intermediate-/poor- versus good-risk patients, we explicitly assessed differences between the intermediate- and poor-risk groups.^{3,7} Outcomes on first-line sunitinib or pazopanib, distribution of molecular subtypes, Angio score, and proportion of $\geq 25\%$ sarcomatoid tumors were all significantly different between the intermediate- and poor-risk groups. Moreover, when we repeated all analyses while comparing IMDC good risk with pooled intermediate/poor risk, the results were not as solid as when considering the 3 groups separately (see [Supplemental Tables 9 and 10](#) in the online version).

Discussion

There is still no optimal way of selecting patients with accRCC for treatment with angiogenesis inhibitors, ICPIs, or the upcoming combinations. Recent trials have suggested IMDC risk group, angiogenic or T_{eff} gene expression, and sarcomatoid dedifferentiation as possible biomarkers, but they all have important limitations.^{4,7,11} In this study, we assessed the correlations between these markers and investigated whether the information they provide could be summarized by a single biomarker, the molecular ccrcc1 to ccrcc4 subtypes. A secondary aim was to characterize the large but heterogeneous IMDC intermediate-risk group on a molecular level.

IMDC good-risk patients had higher angiogenic gene expression, associated with response to sunitinib. This was explained by the overrepresentation of the pro-angiogenic ccrcc2 subtype in the IMDC good-risk group. The predictive value for outcome on sunitinib or pazopanib was similar for molecular subtypes, IMDC risk groups, and angiogenic signature. But more importantly, the ccrcc2 subtype could identify tumors with an angiogenic signature within the IMDC intermediate-risk group and was predictive for outcome on sunitinib and pazopanib in this population. As the majority of patients with accRCC are IMDC intermediate risk, molecular subtypes could contribute to personalized therapy decisions in this population.

When looking at immune gene expression, we found no differences between IMDC risk groups. This is in accordance with responses to ICPIs that were observed across all risk groups in the Checkmate214 trial and with similar T_{eff} -signatures observed in the IMmotion151 trial.^{4,7} However, molecular subtypes showed marked differences in immune gene expression, with ccrcc4 and a subgroup of ccrcc2 tumors displaying immune activation. Therefore, the lack of difference in Immune score between IMDC risk groups hides more pronounced differences in immune phenotype. IMDC good-risk patients mainly

have ccrcc2 tumors, a subgroup of which have an activated immune response. IMDC poor-risk patients mainly have either immune-exhausted ccrcc4 tumors or immune-cold ccrcc1 tumors.¹²

We also found a marked difference between IMDC intermediate- and poor-risk patients on a molecular level. Any comparison of these risk groups should therefore be performed preferentially without pooling IMDC intermediate- and poor-risk patients.

Based on these data, ccrcc1 to ccrcc4 molecular subtypes incorporate in a single marker the information provided by IMDC risk group and expression of angiogenesis and immune response genes. The subtypes were not developed as a marker for a specific therapy and might therefore retain their value in different therapeutic settings and for the development of novel therapeutic options. For example, in the setting of metastasectomy, ccrcc1 and ccrcc4 tumors are at risk for early relapse.¹³ Upon treatment with sunitinib or pazopanib, ccrcc2 and ccrcc3 have favorable outcomes. ccrcc4 tumors can be expected to be the best candidates for ICPIs. As earlier research showed that ccrcc4 tumors exhibit an immune suppressive microenvironment with high levels of myeloid inflammation, they will probably benefit especially from a combination of ICPIs and angiogenesis inhibitor, where the latter suppresses myeloid inflammation.¹² For the immune-cold ccrcc1 tumors, immune sensitizing therapies should be investigated.

Sarcomatoid dedifferentiation has been associated with poor prognosis and outcome on sunitinib or pazopanib.^{19,20} We showed on a large patient series that a cutoff percentage of 25% sarcomatoid dedifferentiation separated tumors with distinct therapeutic sensitivity and molecular characteristics. Tumors with $\geq 25\%$ sarcomatoid dedifferentiation had poor outcomes on first-line sunitinib or pazopanib, low angiogenic gene expression, and were typically IMDC poor risk and ccrcc4 subtype. Contrarily, outcomes and molecular characteristics were similar for tumors with 0% versus 1% to 24% sarcomatoid dedifferentiation. Interestingly, 4 sarcomatoid tumors treated with ICPIs at our institution all responded, whereas outcomes were poorer in 88 patients with 0% to 24% sarcomatoid dedifferentiation.

This study has its limitations. Most importantly, the CIT database only contains a limited number of patients treated with ICPIs, to assess the hypothesized predictive value of molecular subtypes in this context. Two ongoing prospective trials are evaluating this question (Nivoren NCT03013335; Bionikk NCT02960906). Secondly, molecular subtype is currently determined on fresh frozen tissue. To facilitate the classification procedure, a major future aim is to validate the classifier on paraffin-embedded tissue.

In conclusion, molecular subtypes in accRCC can explain differences in IMDC risk group, expression of angiogenic and immune response genes, and sarcomatoid dedifferentiation. Favorable outcomes on first-line sunitinib or pazopanib are predicted by IMDC good risk group, ccrcc2 molecular subtype, and angiogenic gene expression. Molecular subtypes could select patients within the IMDC intermediate risk group for angiogenesis inhibitors, but validation on paraffin-embedded tissue is needed before potential clinical implementation. As molecular subtypes also identify tumors with different immune profiles, where IMDC risk groups do not, they have a potential use in the setting of ICPIs and combination therapies. For sarcomatoid dedifferentiation, a cutoff value of 25% separates tumors with distinct prognosis and molecular characteristics. Tumors with $\geq 25\%$ sarcomatoid dedifferentiation have low angiogenic gene expression and are

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typically IMDC poor risk and ccrc4 subtype. They have poor outcomes on sunitinib or pazopanib, but ICPIs seem a promising option.

Clinical Practice Points

- Metastatic RCC can be treated with angiogenesis inhibitors, ICPIs, or combination therapy. There is currently no reliable way of patient selection. IMDC good risk is associated with better outcome on sunitinib, whereas ICPIs are effective across IMDC risk groups. The majority of patients are intermediate risk, with very heterogeneous outcomes on all therapies.
- ccRCC can be divided into 4 molecular subtypes, ccrc1 to ccrc4. Here, we show that the molecular ccrc2 subtype defines highly angiogenic tumors, explaining the different therapeutic sensitivity of IMDC risk groups. It is enriched in IMDC good-risk patients and can identify patients that are likely to respond to sunitinib or pazopanib within the intermediate-risk group. Immune signature is different between the 4 molecular subtypes, but not between IMDC risk groups.
- Sarcomatoid RCC have poor prognosis and poor outcome on sunitinib and pazopanib. Here, we show that ccRCC with $\geq 25\%$ sarcomatoid component are molecularly different from those with 0% to 24%, whereas there were no differences between those with 0% and 1% to 24%. Sarcomatoid ccRCC ($\geq 25\%$) were enriched for the ccrc4 subtype and had low angiogenic signature and poor outcome on sunitinib or pazopanib. In a small series treated with ICPIs, results were promising.
- Molecular subtypes therefore provide a maximum of information as a single generic biomarker. They are associated with angiogenic and immune signature, IMDC risk group, sarcomatoid dedifferentiation, and outcome on sunitinib and pazopanib. They may be used for patient selection in the future, especially within the IMDC intermediate-risk group.

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Disclosure

B. Beuselinck reports consultancy fees from Amgen, Ipsen, Pfizer, and Novartis and research grants from Bristol-Myers Squibb and Ipsen. The remaining authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental tables accompanying this article can be found in the online version at <https://doi.org/10.1016/j.clgc.2019.05.009>.

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Supplemental Data

Supplemental Table 1		Characteristics of 163 Patients of Whom IMDC Risk Group and Molecular Data Were Available
Characteristic	N	
Total	163	
Male	113	
First-line sunitinib or pazopanib		
Sunitinib	110	
Pazopanib	35	
Median age at start of first-line, y	63	
IMDC risk group		
Good	22	
Intermediate	107	
Poor	34	
ccrcc1 to -4 subtype		
ccrcc1	49	
ccrcc2	70	
ccrcc3	10	
ccrcc4	34	
Angio score		
High	80	
Low	83	
T stage		
T1	28	
T2	19	
T3	109	
T4	4	
Synchronous metastases	86	
Fuhrman grade		
1	0	
2	6	
3	53	
4	104	
Sarcomatoid dedifferentiation		
<25%	146	
≥25%	13	

Abbreviation: IMDC = International Metastatic RCC Database Consortium.

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Supplemental Tables 2 and 3. Differences in Angiogenic and Immune Gene Expression Between IMDC Risk Groups and ccrcc1 to ccrcc4 Molecular Subtypes.

Supplemental Table 2 Angiogenic Gene Expression is Higher for IMDC Good > Intermediate > Poor Risk and ccrcc2 > ccrcc3 > ccrcc1 > ccrcc4 Subtypes

Gene	IMDC Risk Group				ccrcc1 to -4 Molecular Subtype				
	Good	Intermediate	Poor	P Value	ccrcc1	ccrcc2	ccrcc3	ccrcc4	P Value
<i>VEGFA</i>	7.8	5.0	2.8	<.001	3.2	8.5	2.0	2.5	<.0001
<i>VEGFC</i>	0.86	0.92	0.55	.3	0.83	0.92	1.2	0.52	.04
<i>VEGFR1</i>	3.3	1.8	0.10	<.001	1.0	3.3	0.95	0.52	<.0001
<i>VEGFR2</i>	1.6	0.87	0.38	<.001	0.38	1.6	0.61	0.29	<.0001
<i>VEGFR3</i>	1.1	0.83	0.40	<.001	0.64	1.2	0.68	0.36	<.0001
<i>HIF1A</i>	0.81	0.63	0.64	.3	0.81	0.64	2.2	0.50	.008
<i>HIF2A</i>	1.2	0.98	0.49	<.001	0.67	1.3	0.95	0.39	<.0001
<i>PDGFRB</i>	1.5	1.4	1.1	.07	1.4	1.6	1.6	0.89	.008
<i>ANGPTL4</i>	88	70	35	.05	72	103	2.6	27	<.0001

The largest difference is in expression of *VEGFR2*, the most important target of sunitinib and pazopanib (+321% for IMDC Good vs. Poor and +440% for ccrcc2 vs. ccrcc4). Abbreviation: IMDC = International Metastatic RCC Database Consortium.

Supplemental Table 3 Immune Gene Expression is Not Different Between IMDC Risk Groups, but Significantly Higher for ccrcc4 > ccrcc3 > ccrcc1 and ccrcc2

Gene	IMDC Risk Group				ccrcc1-4 Molecular Subtype				
	Good	Intermediate	Poor	P Value	ccrcc1	ccrcc2	ccrcc3	ccrcc4	P Value
<i>PD-L1</i>	1.0	1.0	1.2	.9	0.51	1.2	1.5	1.2	.008
<i>IFN-γ</i>	2.4	2.8	2.0	.5	1.4	2.9	1.2	6.3	.0007
<i>PD1</i>	3.4	6.1	5.3	.5	3.8	5.7	3.4	11.1	.001
<i>PD-L2</i>	1.3	1.6	1.3	.9	0.78	1.8	1.4	2.2	<.0001
<i>LAG3</i>	3.6	5.1	5.5	.5	3.6	5.0	3.2	11	<.0001
<i>CD3G</i>	3.2	3.3	2.2	.3	2.0	3.7	1.9	4.0	.0004
<i>CXCL13</i>	4.3	8.4	9.5	.3	4.9	8.2	2.5	34	<.0001
<i>CXCL9</i>	2.7	3.3	2.6	.9	1.9	4.3	1.8	10	<.0001
<i>CSF1R</i>	3.4	3.3	4.1	.8	2.6	3.8	2.9	3.7	.5
<i>IL10</i>	2.7	2.4	2.3	1	2.4	2.5	2.7	2.4	.9
<i>IL12a</i>	0.55	0.53	0.36	.3	0.35	0.51	1.0	0.57	.05
<i>TNF</i>	0.88	0.86	0.94	1	0.58	0.87	1.4	1.4	.0006
<i>TIM3</i>	1.3	1.1	0.94	.3	0.97	1.3	1.5	0.83	.02
<i>CD8a</i>	4.8	6.3	4.3	.5	3.6	7.4	2.1	10	<.0001

Median fold change is reported and compared with the Kruskal Wallis test. Abbreviation: IMDC = International Metastatic RCC Database Consortium.

Supplemental Tables 5 and 6. Multivariable Cox regression for impact of sarcomatoid dedifferentiation on progression-free and overall survival on first-line sunitinib or pazopanib (n = 366). The following predictors were included in the models: sarcomatoid dedifferentiation (0%-24% vs. $\geq 25\%$), IMDC (Good vs. Inter-

mediate vs. Poor), time of metastases (synchronous vs. metachronous), Fuhrman grade (< 4 vs. 4), T-stage (1-2 vs. 3-4), and age at start of first-line therapy. Molecular subtype and Angio score were not included, as these were only known for a minority of patients. Sarcomatoid dedifferentiation and IMDC risk group remained independent predictors of progression-free and overall survival.

Supplemental Table 4 Characteristics of 366 Patients Treated With First-line Sunitinib or Pazopanib, for Whom Percentage of Sarcomatoid Dedifferentiation was Available

	Non-sarcomatoid ($< 25\%$), n = 344 (%)	Sarcomatoid ($\geq 25\%$), n = 22 (%)
Median age at start, y	64	61
Male	228 (66)	16 (73)
First-line therapy		
Sunitinib	248 (72)	18 (82)
Pazopanib	96 (28)	4 (18)
IMDC risk group		
Good	45 (14)	0
Intermediate	195 (60)	9 (43)
Poor	85 (26)	12 (57)
ccrcc1 to -4 subtype		
ccrcc1	44 (30)	3 (23)
ccrcc2	67 (45)	2 (15)
ccrcc3	9 (6)	0
ccrcc4	26 (18)	8 (62)
Angio score		
High	76 (52)	3 (23)
Low	70 (48)	10 (77)
T stage		
T1	63 (19)	3 (14)
T2	64 (20)	3 (14)
T3	186 (57)	13 (62)
T4	13 (4)	2 (10)
Synchronous metastases	163 (47)	15 (68)
Fuhrman grade		
1	5 (1)	0
2	37 (11)	0
3	143 (42)	0
4	157 (46)	22 (100)

Abbreviation: IMDC = International Metastatic RCC Database Consortium.

Supplemental Table 5 Progression-free Survival

Predictor	Hazard Ratio	95% Confidence Interval	P Value
$\geq 25\%$ sarcomatoid dedifferentiation	2.9	1.8-4.5	$<.0001$
IMDC intermediate risk (vs. good)	1.4	0.9-2.2	.1
IMDC poor risk (vs. good)	2.3	1.5-3.7	.0003

Abbreviation: IMDC = International Metastatic RCC Database Consortium.

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Supplemental Table 6 Overall Survival

Predictor	Hazard Ratio	95% Confidence Interval	P Value
≥ 25% sarcomatoid dedifferentiation	2.3	1.4-3.7	.001
IMDC intermediate risk (vs. good)	1.9	1.2-3.1	.009
IMDC poor risk (vs. good)	3.2	1.9-5.4	<.0001

Abbreviation: IMDC = International Metastatic RCC Database Consortium.

Supplemental Tables 7 and 8. Comparison of ccRCC with 0% and 1% to 24% Sarcomatoid Dedifferentiation.

Supplemental Table 7 IMDC Risk Group and Angio Score do Not Differ Between Tumors With 0% Versus 1% to 24% Sarcomatoid Dedifferentiation

	0%, n (%)	1%-24%, n (%)	P Value
IMDC risk group			.4
Good	18 (17)	3 (8)	
Intermediate	72 (67)	28 (74)	
Poor	17 (16)	7 (18)	
Median Angio score	−0.4	−0.4	.99
ccrcc1 to -4 molecular subtype			
ccrcc1	28 (26)	19 (37)	Numbers too small for Fisher exact test
ccrcc2	55 (55)	14 (27)	
ccrcc3	6 (6)	3 (6)	
ccrcc4	18 (17)	16 (31)	

P values calculated with the Fisher exact test (IMDC) and logistic regression (Angio score).
Abbreviation: IMDC = International Metastatic RCC Database Consortium.

Supplemental Table 8 Expression of Angiogenesis Genes is Similar Between ccRCC With 0% and 1% to 24% Sarcomatoid Dedifferentiation, and Higher Compared With ccRCC With ≥25% Sarcomatoid Dedifferentiation

Gene	Sarcomatoid Component		
	0%	1%-24%	≥ 25%
VEGFA	5.7	4.7	2.7
VEGFC	0.82	0.89	0.58
VEGFR1	1.8	1.9	0.46
VEGFR2	0.89	0.87	0.17
VEGFR3	0.83	0.85	0.38
HIF1A	0.71	0.83	0.47
HIF2A	1.0	0.81	0.29
PDGFRB	1.4	1.5	0.99
ANGPTL4	63	72	32

Median fold change is reported.
Abbreviation: ccRCC = Clear-cell renal cell carcinoma.

Supplemental Tables 9 and 10. The 3 IMDC Risk Groups are Distinctly Different; Therefore, Pooling of Intermediate and Poor Risk Should be Avoided When Possible.

Supplemental Table 9 Comparison of IMDC Good-risk Patients With Pooled Intermediate/Poor Risk			
	IMDC Risk Group		P Value
	Good	Intermediate/Poor	
Outcome on first-line sunitinib or pazopanib			
RR, %	63	41	.08
PFS, mos	25	11	.02
Angio score			
High	77% (17)	45% (63)	.02
Low	23% (5)	55% (78)	
Individual angiogenesis genes, median fold change			
VEGFA	7.8	4.3	.01
VEGFC	0.86	.80	.5
VEGFR1	3.3	1.59	.004
VEGFR2	1.6	.66	.003
VEGFR3	1.1	.72	.02
HIF1A	0.81	.63	.2
HIF2A	1.2	.79	.01
PDGFRB	1.5	1.4	.2
ANGPTL4	88	63	.2
ccrcc1 to -4 molecular subtype			
ccrcc1	14% (3)	33% (46)	.002
ccrcc2	77% (17)	38% (53)	
ccrcc3	9% (2)	6% (8)	
ccrcc4	0	24% (34)	
Sarcomatoid dedifferentiation			
<25%	100% (21)	91% (124)	.14
≥25%	0	9% (13)	

The differences are less pronounced and *P* values are higher than upon comparison of the 3 IMDC risk groups separately.

Abbreviations: IMDC = International Metastatic RCC Database Consortium; PFS = progression-free survival; RR = response rate.

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Supplemental Table 10 Clinical and Molecular Characteristics are Significantly Different Between IMDC Intermediate- and Poor-risk Patients

	IMDC Risk Group, % (n)		P Value
	Intermediate	Poor	
Outcome on first-line sunitinib or pazopanib			
RR, %	46	19	.03
PFS, mos	13	5	.0009
Angio score			
High	50 (54)	26 (9)	.01
Low	50 (53)	74 (25)	
Individual angiogenesis genes, median fold change			
<i>VEGFA</i>	5.0	2.8	.003
<i>VEGFC</i>	0.92	0.55	.2
<i>VEGFR1</i>	1.8	0.10	.02
<i>VEGFR2</i>	0.87	0.38	.004
<i>VEGFR3</i>	0.83	0.40	.0007
<i>HIF1A</i>	0.63	0.64	.7
<i>HIF2A</i>	0.98	0.49	.004
<i>PDGFRB</i>	1.4	1.1	.06
<i>ANGPTL4</i>	70	35	.03
ccrcc1 to -4 molecular subtype			
ccrcc1	33 (35)	32 (11)	.03
ccrcc2	43 (46)	21 (7)	
ccrcc3	6 (6)	6 (2)	
ccrcc4	19 (20)	24 (14)	
Sarcomatoid dedifferentiation			
<25%	95 (100)	75 (24)	.002
≥25%	5 (5)	25 (8)	

P values were calculated with the Fisher exact test (Angio score, ccrcc1 to ccrcc4, sarcomatoid), logistic regression (RR), Kaplan-Meier method (PFS), and Mann Whitney U test (fold change). Abbreviations: IMDC = International Metastatic RCC Database Consortium; PFS = progression-free survival; RR = response rate.