World Journal of Urology (2021) 39:1377–1385
https://doi.org/10.1007/s00345-020-03528-x

TOPIC PAPER

Update on the most promising biomarkers of response to immune checkpoint inhibitors in clear cell renal cell carcinoma

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Received: 20 July 2020 / Accepted: 13 November 2020 / Published online: 2 January 2021

Abstract

In the last few years, the standard of care for metastatic clear cell renal cell carcinoma (mccRCC) has changed dramatically with the emergence of the immune checkpoint inhibitors (ICI): anti-PD(L)-1 used as a monotherapy or as in combination either with an anti CTLA-4 or with an anti-angiogenic molecule (VEGFR tyrosine kinase inhibitor (TKI)). These combinations are now recommended in first line setting for mccRCC, according to the last European recommendations. In the face of these new therapeutic options, the question of selecting the best treatment arises as well as the optimal sequence. Predictive biomarkers are required to guide the therapeutic choice and provide a personalized treatment for each patient. This narrative review will provide an overview of the main predictive biomarkers assessed in mccRCC treatment, with a particular focus on mRNA panel signatures.

Keywords Clear cell renal cell carcinoma · Predictive biomarkers · mRNA signature · Genomic alterations · Tumor microenvironment · Circulating biomarkers

Introduction

The prognosis of clear cell renal cell carcinoma (ccRCC) has been drastically improved over the past decades with the emergence of new treatments: anti-VEGFR tyrosine kinase inhibitors (TKI), mTOR inhibitors, and most recently, immune checkpoint inhibitors (ICI) blocking the PD-1/PD-L1 and CTLA-4 axis [1]. In the last few months, combinations of anti PD-1/anti CTLA-4 or anti PD-1/anti VEGFR TKI have been approved in front-line treatment of metastatic ccRCC (mccRCC) [2–4]. European recommendations have been recently updated to integrate new ICI-based combinations [4]. The patient stratification by risk scores (IMDC and/or MSKCC) is currently used to decide which treatment is more accurate in frontline [2, 4]. Indeed, pembrolizumab (anti-PD-1)—axitinib (TKI) combination is recommended for all risk group based on the KEYNOTE 426 [3, 5] whereas nivolumab (anti-PD-1)-ipilimumab (anti-CTLA-4) combination is recommended only in intermediate/poor risk group [2, 4] Despite clear benefit of these treatments over large populations, there is an urgent need for predictive biomarkers that could guide therapeutic strategy at the individual level. However, to date, no biomarker seems to be strong enough to accurately identify ICI and/or TKI efficacy, since many biomarker-negative patients could be responders to these treatments. We conducted an unsystematic narrative review using PubMed and Google Scholar databases. We used several keywords simultaneously, including *clear cell renal cell carcinoma, immune checkpoint inhibitors, mRNA signatures, genomic alteration and tumor micro environment*. Only studies published in English or French language up to June 2020 were included in the search process. Published trials, published reviews and European Society of Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO) and ASCO-genito-urinary conferences abstracts were tracked. This review will discuss the emerging biomarkers of ccRCC that could be used in the future to help decide the treatment sequence and/or strategy. Among these new biomarkers, we will focus

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on mRNA panel signatures which appear to be the most promising.

Molecular analysis for selecting therapeutic strategy in ccRCC

At the tumor level, genomic approaches have been shown to be useful tools in some solid tumors to comprehensively evaluate the genetic alterations displayed by cancer cells and relevantly establish global genetic characteristics with potential influence on antineoplastic treatment sensitivities and indications [6]. More focused assessment of certain mutations and pathway alterations displayed by ccRCC, already known for having a prognosis signification, could also potentially provide useful information to guide treatment of the disease at different stages [7] (Fig. 1). In line with increasing consideration of the tumor micro-environment and immune contexture, transcriptomic approaches enable to encompass the whole system by providing functional information about changes in gene expression, occurring not only in tumor cells, but also in the surrounding components. Such analysis allows a global assessment of ongoing pro- and anti-tumor processes, notably angiogenesis and immune processes, and can be complementary to genetic [8].

In few tumor types, such as breast cancer, molecular signatures has already been validated for routine practice and represent considerable advances toward personalized medicine [9, 10]. These reflections have led to establish retrospectively and prospectively predictive molecular signatures in ccRCC, along with industrial therapeutic trials of antiangiogenic and immunotherapeutic drugs or within academic research.

Quantitative approaches

Tumor mutational burden (TMB)

Mutations occurring in tumor genetic material can lead to the production of abnormal proteins, resulting eventually in the presentation of immunogenic tumor specific neo-antigen [11]. It has been hypothesized that a higher frequency of non-synonymous mutations in tumor cells, the so called "tumor mutational burden (TMB)", is correlated with a higher rate of neo-antigens production and a higher probability of triggering an adaptive immune response that could be reinvigorated by ICI. The association between TMB and response to ICI was globally demonstrated in a meta-analysis across 27 tumor types [12]. However, in comparison with other tumor types, mccRCC generally displays a lower TMB, on average 1.1 mutations/Mb but a high overall response rate to ICI [12]. In an exploratory analysis of a phase II trial of atezolizumab in mccRCC patients (IMmotion 150), TMB was not associated with progression-free survival (PFS) [13]. In a recent post hoc analysis of the phase III CheckMate 214 trial assessing nivolumab plus ipilimumab efficacy

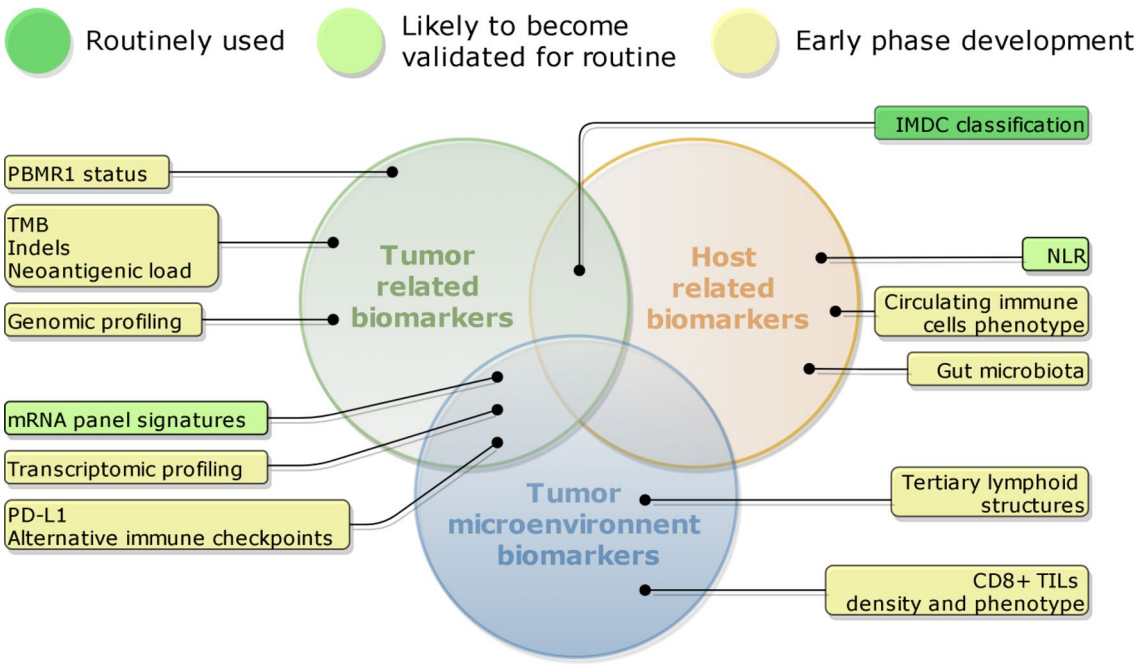

Fig. 1 Validated and emerging biomarkers for mccRCC treatment. *mccRCC* metastatic clear cell renal cell carcinoma, *IMDC* International Metastatic RCC Database Consortium, *NLR* neutrophil-lymphocyte ratio

in mccRCC, TMB was not associated with survival outcome either [14]. Thus, even if TMB is promising in many solid tumors to predict ICI efficacy, it appears disappointing in mccRCC.

Neoantigens load, Indel mutations

A refinement of the TMB analysis is the neoantigen load assessment, considering that only mutations resulting in the production of immunogenic neoantigens would be relevant to predict the potential of an existing adaptive anti-tumor immune response.

Using the TCGA database, Turajlic et al. demonstrated that ccRCC display the highest proportion of indel mutations accounting to their overall TMB compared to other tumor types [15]. Such mutations would potentially generate more neoantigens by frameshifting than single nucleotide variants mutations, and thus may favor sensitivity to ICI in ccRCC despite their low TMB. Accordingly, Voss et al. found a positive association of frameshift indel mutation counts with overall survival (OS) in a cohort of ccRCC treated with anti-PD-1, a similar association was found in patient treated with TKI but did not reach significance [16]. No correlation between neoantigens load, PFS and OS was found in this study. Nevertheless, in a recent post hoc analysis of the ChecKMate 214 study, high indel counts were not associated with survival outcomes for patients treated with nivolumab plus ipilimumab although they were associated with improved PFS and OS in patients treated with sunitinib [14].

Recent data from Labriola et al. suggest that calculation of the neoantigens load of ccRCC, have failed to predict the immunogenicity of tumors because it does not take frequent defects of the antigen presentation machinery into account [17]. Indeed, a high calculated neoantigens load would not result in a highly immunogenic tumor if alterations such as MHC loss of heterozygocity or loss of β-2 microglobulin impair the presentation of putative neoantigens. However, they found more frequent mutations of DNA damage response genes in ICI responders. In addition, mutations occurring in non-exonic regions of tumor DNA can lead to abnormal mRNA and peptide sequence, particularly via alternative splicing, thus generating potential neoantigens that would not have been predicted with the methods described above [18].

Particular genetic alterations

Polybromo 1 (PBRM1) mutation Multicentric genomic analysis of ccRCC pooled in TCGA had led to the identification of PBRM-1 as the second most frequently mutated gene in this tumor type (40–50% of cases) [19]. PBRM1 function is part of the epigenetic regulatory mechanisms of DNA expression and known for its prognostic value independently of validated clinical classifications [20]. Recent histopathological and pre-clinical data suggest that PBRM1 loss of function (LOF) associates with a highly angiogenic but non-immunogenic tumor phenotype [21]. In this context, its relationship with sensitivity to ccRCC treatments has been investigated as a part of biomarkers ancillary studies but its predictive role still remains debated.

In a retrospective analysis of COMPARZ and RECORD-3 trials, PBRM1 mutation status was found to correlate with better outcome of mccRCC with sunitinib or pazopanib treatment [22]. PBRM1 LOF was also found to be positively associated with clinical benefit with nivolumab treatment in a cohort of 35 mccRCC; this was confirmed in a validation cohort of 63 mccRCC with anti-PD-1/PD-L1 monotherapy or combined with anti-CTLA4 ICI [23]. Similar correlation with ORR with nivolumab was found in a retrospective analysis of the CheckMate 025 data [24]. Nonetheless, contradictory results were found in the exploratory biomarker analysis of IMmotion 150: PBRM1 alteration was correlated with better PFS with sunitinib treatment but no significant association was found for atezolizumab (anti-PD-L1) treatment. Moreover PBRM1-mutated patients exhibited poorer PFS with atezolizumab when compared with sunitinib and atezolizumab plus bevacizumab [13]. Braun et al. recently revealed that mccRCC tumors highly infiltrated with CD8 + T cells are depleted of favorable PBRM1 mutations and enriched for deleterious chromosomal losses of 9p21 [25].

mRNA panel signatures

Main mRNA panel signatures developed or designed within prospective trial in ccRCC are summarized in Table 1 (adapted from [26]).

IMmotion 150 and 151

The randomized phase II clinical trial IMmotion 150 compared atezolizumab, sunitinib and atezolizumab-bevacizumab as first line treatment of mccRCC. It included a biomarkers analysis, exploring the predictive value of TME using transcriptional analysis. The authors designed three gene panels for mRNA quantification, the Angio, T_{eff} and Myeloid inflammation signatures. Combination of these panels provided insight of the sensitivity of tumor to the three regimens according to their TME features. Patients displaying an $Angio^{High}$ signature, associated with high vascular density, showed improved overall response rate (ORR) compared to $Angio^{Low}$ (46 versus 9% respectively) when treated with sunitinib. Conversely, patients displaying an $Angio^{Low}$ signature benefited more in terms of PFS from the atezolizumab-bevacizumab combination compared

Table 1 Molecular signatures under investigation in mccRCC (adapted from [26])

Signatures	Study Design	Number of patients	Genes involved in the signature	Treatment studied	Predictive value of response	
					ITK	ICI
CIT [30]	Retrospective	52 (exploratory cohort) 47 (validation cohort)	Inflammation, myeloid activation, migration cells, Th1/Th2 polarization, T cells, TGFb, IL10, IL17	Sunitinib	YES	Ongoing (BION-IKK trial [32])
IMmotion 150/151 [13, 27]	Randomized, prospective, phase II and III	305 (IMmotion 150) 915 (IMmotion 151)	Angiogenesis, immunitary response, IFNg, inflammation, myeloid cells	Atezolizumab ± Bevacizumab Sunitinib	YES	YES
Javelin Renal 101 [29]	Randomized, prospective, phase III	886	Immunitary response (TcR signalisation, T cells activation and proliferation, cell differenciation) chemokines, NK cells	Avelumab + Axitinib Sunitinib	NO	YES
CheckMate 214 [14]	Randomized, prospective, phase III	213	Angiogenesis, inflammation, epithelial-mesenchymal transition	Nivolumab + Ipilimumab Sunitinib	YES	YES

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to sunitinib. Patients displaying a T_{eff}^{High} signature, associated with a high CD8 + T cells infiltration, showed improved ORR compared to T_{eff}^{Low} (49% versus 16% respectively) when treated with atezolizumab-bevacizumab. $Myeloid^{High}$ tumors, characterized by increased myeloid inflammation, showed poor PFS with the three regimens. Nonetheless, patients displaying an addition of T_{eff}^{High} and $Myeloid^{High}$ signatures had increased PFS under atezolizumab-bevacizumab combination (25 months) compared to atezolizumab alone or sunitinib (2 and 7 months respectively). These observations were confirmed in a larger cohort within the subsequent phase III study, IMmotion 151 [27].

JAVELIN Renal 101

The phase III JAVELIN Renal 101 trial compared avelumab, an anti-PD-L1 ICI, combined with axitinib to sunitib as treatment of frontline mccRCC [28]. Similar mRNA analyses to those performed in IMmotion trials also found an increased efficacy of sunitinib in $Angio^{High}$ tumors compared with $Angio^{Low}$ in terms of PFS. In T_{eff}^{High} patients, results suggested an increased efficacy of the avelumab-axitinib combination compared to sunitinib but did not reach statistical significance. A novel immune-related mRNA panel signature related to T, NK-cell activation and IFNγ signaling was associated to better PFS with avelumab-axitinib treatment when positive compared to negative tumors (15.2 versus 9.8 months). This signature was further validated in the independent cohort of the phase I JAVELIN Renal 100 study [29].

CheckMate 214

The phase III CheckMate 214 trial compared nivolumab plus ipilimumab combination to sunitib as treatment of advanced RCC. It included biomarkers analysis along those described in JAVELIN and IMmotion studies. $Angio^{High}$ tumors showed increased ORR and PFS under sunitinib treatment whereas $Angio^{Low}$ showed higher ORR under nivolumab plus ipilimumab treatment. There was no significant difference between arms associated with the T_{eff} signature but one should note that in contrast to Javelin and IMmotion study, PD-1/PD-L1 axis blockade was not combined with an antiangiogenic drug. Transcriptomic data yielded on 213 individuals revealed that prolonged PFS under nivolumab plus ipilimumab treatment was associated with higher expression of a gene set related to inflammatory response and epithelial mesenchymal transition, defining an additional mRNA panel signature [14].

Tumor Identity Card (CIT)–BIONIKK

The French Tumor Identity Card (CIT) consortium yields large scale molecular data on various tumor types to promote precision medicine. A 35-gene expression signature has been developed by unsupervised clustering of transcriptomic data

from 53 primary resected ccRCC tumors and validated in an external cohort, to predict which patients would benefit from sunitinib in frontline of mccRCC [30]. Four molecular groups (ccrcc1 to 4) were thus determined that significantly associated with differential outcome (ORR, PFS and OS) under sunitinib treatment. Group 4 (ccrcc4) was characterized by a strong inflammatory, Th1-oriented, but immunologically suppressed microenvironment whereas ccrcc1 group displayed very low T cell infiltration and could be described as "cold" tumors. Both subgroups exhibited poor ORR, PFS and OS under sunitinib treatment. Group 3 tumors displayed no hypoxia-induced cellular response and had a similar profile to normal kidney; ccrcc2 tumors which were a mixed group of tumors mainly characterized by a pro-angiogenic profile and some of them having a T effector profile. These two groups had significantly better ORR, PFS and OS under sunitinib [30]. Verbiest et al. characterized more clearly ccrcc2 tumors and found a strong overlap between IMDC good risk group, $Angio^{High}$ tumors and ccrcc2 tumors. 77% of IMDC favorable prognosispatients had a ccrcc2 and $Angio^{High}$ tumor [31].

Based on this data, we launched the ongoing randomized phase 2 trial BIONIKK (NCT02960906) which is evaluating the efficacy of nivolumab alone or in combination with ipilimumab, or TKI (sunitinib or pazopanib at investigators choice) in frontline mccRCC [32]. Treatment allocation is based on molecular classification by the CIT signature test assessed in frozen tumors for each patient enrolled. Patients classified in the ccrcc1 and 4 subtypes were randomized to receive either nivolumab alone or in combination with ipilimumab, given their assumed resistance to sunitinib in the preliminary work by Beuselinck et al. [30]. Patients classified in the ccrcc2 and ccrcc 3 subtypes are randomized to receive nivolumab plus ipilimumab combination or TKI based on the assumed good outcomes with sunitinib. The study has randomized 200 patients and results will be reported in late 2020.

Histopathological and cellular biomarkers

PD-L1

Programmed-Death Ligand 1 protein is found in 10–25% of tumor cells in ccRCC and identified as a biomarker for worse prognosis [33].

If in some solid tumors such as non-small cell lung cancers, PD-L1 high expression is associated with response to ICI, it seems to not be the case in ccRCC. In the ChecKMate 025 evaluating nivolumab versus everolimus, the hazard ratios (HR) for death were similar between PD-L1 positive and negative populations (HR = 0.79 (95%CI 0.53–1.17) and HR = 0.77 (95% CI 0.60–0.97) respectively) [34]. In the KEYNOTE 426 and ChecKMate 214 studies, an OS benefit was found regardless of the PD-L1 status [2, 5]. In the JAVELIN Renal 101 study, PD-L1 positive status (immune cells ≥ 1%, 60% of patients) was associated with a worse PFS in the sunitinib arm but not in the avelumab plus axitinib arm, confirming that PD-L1 status is rather a prognostic biomarker than a true predictor of response to ICI [28].

Multiple biases may interfere with the interpretation of PD-L1 expression. First of all, the scoring method used to calculate PD-L1 varies across studies: in the KEYNOTE 426, a combined positive score (CPS) score was used (all $PD\text{-}L1^+$ cells divided by number of tumor cells × 100), whereas only $PD\text{-}L1^+$ tumor cells (TC) were counted in the ChecKMate 214 study and only $PD\text{-}L1^+$ immune cells (IC) were counted in the JAVELIN RENAL study [2, 3, 28]. Of note, in the ChecKMate 214, PD-L1 status was recently reassessed by the CPS and similar results were found [35]. In an ancillary analysis of the NIVOREN phase II trial, evaluating nivolumab in a real-world setting in 720 mccRCC, PD-L1 TC+ (≥ 1%) was associated with worse OS (HR 1.51, 95%CI 1.06–2.15, $p = 0.02$) whereas a trend was seen for PD-L1 IC + (HR = 1.34, 95%CI 0.95–1.88, $p = 0.09$) [36].

In addition, there is a heterogeneous distribution of PD-L1 across tumor cells and/or metastasis which means that results may be pathologist-dependent and tumor sample-dependent [37, 38]. Finally, various anti-PD-L1 antibodies clones (22C3, 28–8, SP142, SP263) are used for immunostaining in different platforms. These biases explain the complexity of PD-L1 status assessment and strengthen the need for standardisation in the staining and calculation methods.

Tumor microenvironment

Tumor microenvironment (TME) is defined as the surrounding tissue of tumor cells which is composed with immune cells (T and B lymphocytes, natural killer cells, myeloid cells…) and stromal cells including endothelial and fibroblasts. This ecosystem regulates all aspects from tumor development, progression to primary and secondary resistance to treatment. Multiple techniques (immunohistochemistry (IHC), flow cytometry, mRNA signatures…) allow to identify all cell subtypes existing in the TME and to correlate with treatment response and resistance mechanisms. Among all type of cells in the ccRCC TME, T lymphocytes are the most reported as potential predictor of ICI (in)-efficacy. CD8 + TILs are associated with a better prognosis in most of solid tumors, but not in ccRCC [39]. A translational study of NIVOREN found that the highest density of CD8 + T cells in the invasive margin (but not in the tumor core) was associated with worse PFS (HR = 3.96, 95%CI 1.84–8.51, $p = 0.0001$) and worse OS (HR = 2.42, 95%CI 0.99–5.95, $p = 0.0451$) [36]. Nevertheless, these results must

be taken with caution since only 7 patients out of 283 were scored as the highest density (score 3). When we combined intermediate and high score (2 and 3), no statistical differences were found compared to scores 0–1 in terms of PFS ($p=0.2$) and OS ($p=0.6$). In the same study, the densities of CD3 or CD20 T cells were not associated with outcomes.

Further studies conducted by Giraldo et al., assessed the role of the CD8 + T cells distinct phenotypes in addition to their density. In a cohort of 40 localized ccRCC, three different immune profiles were identified: an immune-regulated profile with nonfunctional CD8 T cells (expressing PDL-1, LAG-3 and TIM-3), an immune-active profile with LAG-3-negative CD8 T cells and an immune-silent profile with poor T cells infiltration. The immune-regulated profile associated with more aggressive tumors, inflammation and worse prognosis [40]. In an ancillary analysis of the CheckMate 010 study evaluating nivolumab, the authors found that patients with high percentage of PD-1$^+$TIM-3$^-$LAG-3$^-$CD8$^+$ TILs seemed to be associated with a better response rate (45.8 versus 19.6%, $p=0.001$), a clinical benefit and a better PFS (9.6 versus 3.7 months, $p=0.003$) [41]. In the NIVOREN ancillary program we did not find any association between TIM-3 and/or LAG-3 and outcomes (PFS or OS) under nivolumab [42]. Braun et al. recently confirmed in 219 patients treated with anti-PD-1 through clinical trial that CD8$^+$ TILs density was not correlated with clinical benefit, neither with the 3 different immune phenotypes: (CD8$^+$)-infiltrated, immune-desert and immune-excluded [25].

Circulating biomarkers

Circulating T cells

The immune response is mediated by tumor and TME immune infiltration but may need a participation of the circulating immune cells [43]. Unfortunately, only few works were reported on the impact of the circulating immune cells phenotypes on outcomes with anti-PD-1 in mccRCC.

In the immune-monitoring part of the ancillary analysis of the NIVOREN study, authors explored the immune circulating cells in 44 patients treated with nivolumab and their association with treatment response and toxicity. Patients who had a primary resistance had low levels of B cells and CD4$^+$ T cells and high levels of CD244$^+$ neutrophils and CD244$^+$ CD4$^+$ T cells and CD8$^+$ T cells. CD244 is an immune checkpoint highly expressed in exhausted T cells and in an immunosuppressive subgroup of neutrophils. Conversely, high level of non-switched B cells was associated with better PFS and OS. In addition, higher proportion of CD8$^+$PD-1$^+$CD5^{high} and a lower proportion of CD4$^+$PD-1$^-$ T cells were associated with increased toxicity of nivolumab.

Analyses by mass spectrometry of circulating immune cells phenotypes from patients included in the BIONIKK study are planned and may add new insight on the predictive impact of these cells for patients treated with ICI [32].

NLR

Neutrophil to lymphocytes ratio (NLR) is defined as the neutrophils count divided by the lymphocytes count. It is assumed to be a balance between tumor inflammation and tumor immunity. It is well known that a high level of neutrophils is associated with secretions of pro-tumoral molecules (oxygen reactive species, arginase, inflammatory cytokines) and that a low level of lymphocytes is associated with an alteration of anti-tumoral response, CD8$^+$ cytotoxicity and helper CD4$^+$ T cells properties. Thus there is a rational for choosing NLR as a biomarker of ICI response. Many studies have already confirmed the negative prognostic value for NLR, regardless of the type of tumor or chosen treatment [44]. However, since NLR threshold is cohort-dependent, finding a cut-off is a source of bias [45]. Thus, NLR variation could be a simple way to overcome the "optimal cut-off" limitation. In mccRCC, several teams reported results using the NLR variation. In a study by Lalani et al., a 25% increase of NLR in the first 6 weeks of anti-PD-11 treatment was associated with worse PFS and OS [46]. Our team has recently published similar results: in multivariate analysis, any increase of NLR in the first 6 weeks of anti-PD-1 treatment was associated with worse PFS and OS [47]. Other studies are needed to determine if the NLR variation is just a prognostic factor or could specifically predict ICI response.

Other biomarkers of interest

List of currently investigated biomarkers of ICI response is endless, either coming from TME such as the presence of TLS which has recently been shown to be a predictor of ICI response in sarcoma [48]; or related to the host such as gut microbiota which is under intensive research [49].

Nevertheless, promising data on biomarkers developed for other solid tumors may not apply to ccRCC, which means that we still need tumor-type dedicated trial to confirm them.

Clinical practice applicability

To sum up, mRNA panel signatures appear as the most likely biomarkers to guide frontline strategy in the coming years whereas NLR would help monitoring therapeutic response after treatment initiation (Fig. 2). Regarding their cost and

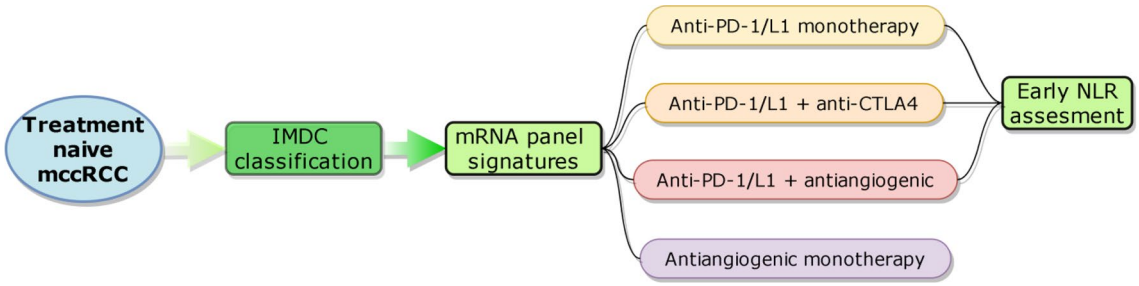

Fig. 2 Proposed algorithm for upcoming therapeutic decision in frontline mccRCC treatment. *mccRCC* metastatic clear cell renal cell carcinoma, *IMDC* International Metastatic RCC Database Consortium, *NLR* neutrophil-lymphocyte ratio

practical applicability, NLR relies on basic biological measures while mRNA panel signatures require more sophisticated technics and additional fresh tumor sample. Nonetheless mRNA quantification of small gene panels is already widely performed for other tumor types, such as breast or non-small cell lung cancers, and seems thus reasonably feasible in cancer care centers or dedicated facilities [6]. Moreover, the additional health expenditure of such analysis could be more than offset if they prevent spending large amount of money in non-optimal immunotherapies. There is still a lack of standardization between mRNA panels although they are already broadly overlapping.

From a clinical and pragmatic point of view, phenotype analysis of circulating immune cell by flow cytometry or mass spectrometry is an appealing method to assess biomarkers of response to IO-based therapies for further reasons: first, blood collection is non-invasive and could be repeated many times during treatment course, allowing monitoring response. Second, preliminary results in some patients with mccRCC included in the NIVOREN trial are very promising [50].

To the best of our knowledge, the biomarker-based randomized phase II trial BIONIKK is the only prospective trial specifically dedicated to find biomarkers of response to TKI or immune checkpoint inhibitors alone or in combination and including transcriptomic analyses as well as circulating immune cells phenotyping. BIONIKK will so provide the strongest answers to combination of biomarkers [32]. Other trials such as FRACTION-RCC (NCT02996110) or PDIGREE (NCT03793166) are more focused on the optimal sequences of therapies but will give us some important clinical insights. FRACTION-RCC (Fast Real-Time Assessment of Combination Therapies in Immuno-Oncology) study is an open-label, randomized phase II trial utilizing an adaptive design to test a variety of immune-oncology combination therapies rapidly. In this adaptive design, there are currently a number of treatment arms: nivolumab and ipilimumab; nivolumab and relatlimab; nivolumab and BMS-986205; and, nivolumab and BMS-813160. The primary outcomes are objective response rate, duration of response, and progression-free survival. PDIGREE is a phase III trial lead by the Alliance cooperative group evaluating nivolumab alone vs nivolumab-cabozantinib in patients with non-complete response or progression of disease at 3 months after an induction with nivolumab-ipilimumab. The primary endpoint is overall survival.

Conclusion

Renal cell carcinoma is a complex entity, with several different clinical and biological features. A greater understanding of the cancer biology has led to a growing number of therapeutic options including anti-VEGFR tyrosine kinase inhibitors, mTOR inhibitors and immune checkpoint inhibitors (ICI): anti PD-1 and anti PD-L1 used as monotherapy or as a combination with anti CTLA-4 or anti angiogenic therapies. Treatment with TKI and/or ICI has drastically improved the survival outcomes of mccRCC patients. In the face of these multiple treatment possibilities, physicians are still lacking predictive biomarkers to guide the therapeutic choice and to reach the optimal therapeutic sequence. Among the different biomarkers, mRNA panel signatures seem to be the most likely to appear in the upcoming algorithms for therapeutic decision. Nevertheless, analysis of immune circulating cells, TME cells and microbiota could also be useful predictive tools. With all these findings, we hypothesize that a single biomarker will not be strong enough to guide the choice of treatment but we will rather need an integrative combination of biomarkers reflecting the tumor as well as its microenvironment and the host. This strategy is at the center of the BIONIKK study whose first results are shortly expected.

Author's Contribution IP: data collection, data analysis, manuscript writing and editing. JN: data collection, data analysis, manuscript writing and editing. AS: data analysis, manuscript editing. SO: data analysis, manuscript editing. YAV: project development, data analysis, manuscript writing and editing.

Funding NA.

Compliance with ethical standards

Conflict of interest S. Oudard: honoraria for advisory board from BAYER, PFIZER, BMS, MDS, SANOFI, ASTELLAS, JANSSEN, NOVARTIS, ROCHE COMPANY. Y.A. Vano: honoraria for advisory board from PFIZER, BMS, MSD, MERCK, NOVARTIS, ROCHE COMPANY SANOFI, ASTELLAS, JANSSEN. Other authors have no conflict of interest to declare.

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