Current Oncology Reports (2023) 25:671–677
https://doi.org/10.1007/s11912-023-01395-4

Current Status of Predictive Biomarker Development in Metastatic Renal Cell Carcinoma

Giuseppe Fotia[1] · Marco Stellato[1] · Valentina Guadalupi[1] · Pierangela Sepe[1] · Melanie Claps[1] · Patrizia Giannatempo[1] · Achille Bottiglieri[1] · Alessandro Rametta[1] · Ida Taglialatela[1] · Chiara Vela[1] · Giuseppe Procopio[1] · Elena Verzoni[1]

Accepted: 14 February 2023 / Published online: 31 March 2023

Abstract

Purpose of Review In this review, we analyze the current state of research in development of new biomarkers that may be useful in managing metastatic renal cell carcinoma (mRCC) setting.

Recent Findings Combining tumor-based biomarkers (gene expression profile) and blood-based biomarkers (ctDNA, cytokines) would be helpful in acquiring information regarding RCC and might be significant in the decision-making process.

Summary Renal cell carcinoma (RCC) is the sixth most frequently diagnosed neoplasm in men and tithe in women, making it responsible for 5% and 3% of all diagnosed cancers respectively. Metastatic stage represents a non-negligible percentage at diagnosis and is characterized by poor prognosis. Despite clinical features and prognostic score could guide clinicians in therapeutic approach of this disease, biomarkers predictive of response to treatment remain an unmet need.

Keywords Metastatic renal cell carcinoma · Tumor biomarkers · Immuno-oncology · VEGF-TKIs · Molecular signatures

Introduction

Renal cell carcinoma (RCC) is the sixth most frequently diagnosed neoplasm in men and tithe in women, making it responsible for 5% and 3% of all diagnosed cancers respectively [1]. Despite the increase in early diagnoses, metastatic disease continues to account for about one-third of all new cases [2]. The improvement of knowledge about biological and molecular characteristics of RCC has led to the development of new drugs such as vascular endothelial growth factor tyrosine kinase inhibitors (VEGF-TKIs) and immune-checkpoint inhibitors (ICI) that changed profoundly the prognosis of metastatic disease compared to the past; nevertheless, overall prognosis remains poor with a 5-year survival rate of less than 20%, even if the outcome depends on several prognostic features [3].

Contradictory results from adjuvant trial and the different responses observed across metastatic patients treated with

✉ Marco Stellato
marco.stellato@istitutotumori.mi.it

Giuseppe Fotia
giuseppe.fotia@istitutotumori.mi.it

Valentina Guadalupi
valentina.guadalupi@istitutotumori.mi.it

Pierangela Sepe
pierangela.sepe@istitutotumori.mi.it

Melanie Claps
melanie.claps@istitutotumori.mi.it

Patrizia Giannatempo
patrizia.giannatempo@istitutotumori.mi.it

Achille Bottiglieri
achille.bottiglieri@istitutotumori.mi.it

Alessandro Rametta
alessandro.rametta@istitutotumori.mi.it

Ida Taglialatela
ida.taglialatela@istitutotumori.mi.it

Chiara Vela
chiara.vela@istitutotumori.mi.it

Giuseppe Procopio
giuseppe.procopio@istitutotumori.mi.it

Elena Verzoni
elena.verzoni@istitutotumori.mi.it

[1] Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Giacomo Venezian 1, Milan, Italy

ICI-TKI or ICI-ICI combinations reveal the heterogeneity and the complexity of RCC. Despite clinical features and prognostic score could guide clinicians in the therapeutic approach to RCC, biomarker predictive of response to treatment remains an unmet need.

Different biomarkers are currently under investigation and include histology and immunohistochemistry features, genomic status, mutation status, transcriptomic signatures, cell type abundance in tumor microenviroment, and gene set enrichment analysis.

A biological marker (biomarker) as defined by the National Institutes of Health (NIH) Biomarkers Definitions Working Group is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [4]. An ideal biomarker should be easily measurable and readily accessible and should help sustain risk stratification process and prognostic evaluation [5].

In this review, we analyze the current state of research in development of new biomarkers that may be useful from a predictive point of view in the metastatic RCC (mRCC) treatment.

Tissue-Based Biomarkers

Gene Expression Signatures

Molecular signatures are defined as genes, proteins, mRNA transcripts, genetic variants, or other variables useful as markers for a cell or a tissue that can be used for diagnostic, prognostic, or therapeutic purposes [6].

Exploratory analysis of gene expression signatures and transcriptomic analyses from patients enrolled in IMmotion150 and IMmotion151 revealed different molecular subsets in mRCC patients.

McDermott et al. analyzed molecular biomarkers and their association with clinical outcomes from patients enrolled in IMmotion150, a randomized phase 2 study evaluating first line treatment with atezolizumab alone or in combination with bevacizumab compared to sunitinib alone having progression free survival (PFS) in the intention to treat (ITT) population and programmed death-ligand 1 (PD-L1) expression on immune cells (IC) analyses as co-primary endpoints. They reported that expression of angiogenesis pathways was higher in favorable risk patients according to Memorial Sloan Kettering Cancer Clinic (MSKCC) score for mRCC whereas T-effector gene signature expression was higher in intermediate-/poor-risk patients and in tumors having sarcomatoid features [7••].

IMmotion151 is a multicenter phase 3 randomized trial with the same treatments arms of IMmotion150, evaluating PFS in PD-L1 positive population and overall survival (OS) in ITT population. Motzer et al. performed integrative multiomics analyses of patients enrolled in this study, evaluating outcomes among the various subsets of study population, according to cancer predominant molecular characteristics [8, 9].

Using non-negative matrix factorization, 7 distinct clusters have been generated:

Cluster 1, angiogenic/stromal, tumors enriched of neoangiogenic-related genes and with a high expression of stroma-specific genes such as transforming growth factor β (TGFβ), WNT, and NOTCH signaling pathway.
Cluster 2, angiogenic with predominantly neoangiogenic features.
Cluster 3, tumors with low expression of angiogenesis and immune genes with an increased expression of genes associated with complement system; the overexpression of these confers worse prognosis [10, 11].
Cluster 4, tumors characterized by low expression of vascular-related genes and enrichment of cell-cycle transcriptional programs (G2M, MYC), high expression of PD-L1, and with the highest tumor-infiltration grade by immune cells across all clusters; this subset has been defined as T-effector/proliferative.

The two other proliferative clusters are cluster 5 proliferative and cluster 6 stromal/proliferative.

The last group was represented by cluster 7 characterized by an augmented expression of snoRNAs.

In this analysis, a correlation between clusters and risk stratification groups (MSKCC/IMDC) was observed; prevalence of angiogenic clusters was observed in the favorable risk group; conversely, the poor-risk group was enriched with the other clusters.

Patients in clusters 1 and 2, angiogenetic clusters, showed a better prognosis in both treatment arms particularly with sunitinib as deducible from their pathogenetic characteristics. Patients in clusters 4 and 5 had the greatest benefit in terms of overall response rate (ORR) and PFS in the ICI plus angiogenesis inhibitor arm (atezolizumab plus bevacizumab), implying greater immunogenicity of these particular subgroup.

Clear cell renal cell carcinoma (ccRCC) with sarcomatoid features (ccRCC-Sarc) had lower expression of hypoxia and neoangiogenesis-related genes compared with their non-sarcomatoid counterparts, exhibiting a proliferative molecular phenotype and a higher expression of PD-L1. These characteristics could explain the better response shown with ICIs rather than with VEGF TKI monotherapy in ccRCC-Sarc.

This analysis also highlighted the association of certain gene mutations with these clusters; for example, mutated PBRM1 tumors have shown an increased expression of genes related to angiogenesis; on the other hand, mutations

of CDKN2A/B and TP53 were prevalent in clusters 4, 5, and 6, subpopulations characterized by a worse prognosis.

These fascinating findings can be considered as novel research field aimed to address the most appropriate treatment to patients. Indeed, according to the reported analysis, good risk patients would be more sensitive to anti-angiogenesis TKI whereas immuno-oncology (IO) would be more suitable for intermediate-/poor-risk patients and ccRCC-Sarc. These findings might explain the different survival advantages for good risk patients treated with ICI-TKI compared to intermediate-poor-risk patients, as recently reported from major pivotal trials [12••].

Tertiary Lymphoid Structures

Among the immune signatures, tertiary lymphoid structures (TLS) are emerging as a promising tissue biomarker. TLS are ectopic lymphoid organs that develop in non-lymphoid tissues at sites of chronic inflammation, including tumors. As reported by Catherine Sautès-Fridman et al. [13], tertiary lymphoid structures (TLS) are highly express in RCC, and data reported by Meylan et al. suggest that the correlation between TLS + and PFS is stronger for patients treated with nivolumab than nivolumab plus ipilimumab [14].

At ESMO 2022, first ancillary analyses from BIONIKK, a randomized phase II trial, identified TLS > 2 as predictor of response to nivolumab and nivolumab plus ipilimumab whereas Ki67 expression and PD1 + cell density associated with higher response rate and PFS [15].

Chromatin Remodeling-Related Genes

Of particular interest in the context of genomic profiling is the evaluation of genes taking part in chromatin remodeling process such as PBRM1, BAP1, and SETD2, all of which are localized in chromosome 3 that is often involved in RCC development [16, 17].

PBRM1 encodes for polybromo 1, a protein which plays a fundamental role in regulation of cellular proliferation and differentiation [18]; this gene is altered in about 30–40% of patients [19]; recent studies have suggested a possible favorable prognostic effect when this gene is altered and might correlate a better response to antiangiogenic and immune treatment [20].

BAP1 mutations represent 5–16% of all mutations in ccRCC [19]; this is a two-hit tumor suppressor gene; alterations of this gene have been evaluated in COMPARZ cohort whose results showed a lower PFS and OS in patients carrying this mutation and treated with VEGF-TKIs compared to BAP1 wild-type population; furthermore, this alteration has been included in the new model of prognostic stratification that combines MSKCC classification with genomic features [21].

SETD2 is involved in splicing and transcription [22], when altered is often associated with PBRM1 mutations and its presence correlates with higher risk of disease recurrence and shorter disease-free survival (DFS) [16].

Further studies will evaluate the use of these molecular signatures to define the best therapeutic choice for every patient.

PD-L1 Expression

PD-L1 is the principal ligand of programmed death 1 (PD-1), a receptor that is expressed on several immune cells such as B cells, T cells (both CD4 + and CD8 +), natural killer T cells, monocytes, and dendritic cells. Their interaction is fundamental in maintaining physiological immune tolerance and immune exhaustion, preventing autoimmune processes and exaggerated tissue damage during chronic infections.

The association between increased PD-L1 in RCC and presence of negative prognostic features has been evaluated by several studies, and in particular, the aberrant expression of this molecule on cancer cells correlates with adverse factors such as higher transfer ratio of lymph glands, tumor necrosis, higher tumor node metastasis (TNM) stage, tumor size, and higher Fuhrman grade [23–26].

In ICI-ICI treated patients, survival advantage and ORR were independent from PD-L1 expression whereas PFS was longer in patients with PD-L1 expression greater than 1%. Similarly, ICI-TKI combination confers survival advantage and response, regardless from PD-L1 expression [27].

These results do not allow to consider PD-L1 as a potential predictor of response to IO-based treatments; it could rather be a prognostic factor, considering the benefits in terms of overall survival in this subpopulation. Other limitations in using this molecule as a biomarker are related to the absence of a standardized procedure for its measurement and tumor heterogeneity, as the expression of PD-L1 can be different based on tumor site (primary versus metastatic) and for its occasional focal expression, which could make its recognition challenging [28, 29].

Tumor Mutational Burden

Tumor mutational burden (TMB) is a genetic feature that can be detected investigating tumor tissue genome; it is measured as somatic, non-inherited, mutation single nucleotide variations (SNVs), and insertion/deletions (indels) per megabase (mut/Mb).

Tumors with a major TMB often harbor mutations concerning genes that play a key role in repairing DNA damage such as mismatch repair genes and are associated to response to ICIs in several tumors.

RCC has a low mutational burden, albeit with differences between various histotypes; in particular, chromophobe RCC

has the lowest amount of mutation while ccRCC and papillary RCC are comparable, with an average of 1.1 mutations/Mb [30, 31].

Despite the low TMB, kidney cancer benefits significantly from treatment with ICIs, similarly to other tumors with high TMB [32]. A possible explanation is that the most frequent mutations found in RCC are indels that could cause frameshift which can subsequently lead to the generation of large amounts of neoantigen, as highlighted in the work of Turajlic et al. in which was found a positive correlation between production of specific neoantigens and increased expression of antigen presenting genes leading to augmented activation of CD8-positive T lymphocytes [33, 34].

To date, TMB is not used in clinical practice in RCC mostly because of high costs and little availability; however, as previously stated, its use as biomarker is still debatable; further studies on this topic are necessary; in this regard, phase II NIVES study could provide useful data on how TMB might be useful in selecting patients who are most likely to respond to therapies based on tumor mutational status [35].

Blood-Based Biomarkers

Soluble Factors

Soluble factors (SF) are molecules that partake in various biochemical processes such as cell proliferation and differentiation. These include VEGF, vascular cell adhesion molecule (VCAM-1), interleukines (IL-6, IL-8), interferon-gamma (IFNγ), and tumor necrosis factor-alpha (TNFα); numerous of these mediators play a crucial role in signal transmissions between cancer cells and surrounding microenvironment.

Modifications of plasma cytokines and circulating angiogenic factors (CAFs) in patients treated with pazopanib have been investigated within the PIPELINE study, a prospective translational trial, which showed a possible clinical usefulness in evaluating baseline cytokine levels and their trend during therapy. Low pre-treatment levels of molecules such IL-6, IL-8, hepatocyte growth factor (HGF), and osteopontin were associated with better tumor response; conversely, high levels of SDF-1 and VEGF-A were associated with progressive disease (PD) [36]. These results imply a possible important role in the evaluation of these molecules to individualize treatment even more.

Recently, Simonetti et al. analyzed the levels of 507 soluble molecules in mRCC patients treated with nivolumab. After multiplicity adjustment, receptor activator of nuclear factor κ B (RANKL) was the only one that retained the statistically significance. Indeed, RANKL levels were higher in primary refractory patients compared to patients who achieved stable disease response (SD) or partial response (PR). On the other side, PFS and OS were longer in patients with low RANKL levels [37].

Receptor activator of nuclear factor κ B (RANK), RANKL, and decoy receptor, osteoprotegerin (OPG), in physiological conditions, helps regulate bone homeostasis and immune system.

In bone metastases (BM) from different neoplasms, RANKL is overexpressed and its amplification causes osteoclasts activation, resulting in amplified bone resorption [38].

Moreover, RANKL has a chemotactic activity inducing migration of cells expressing RANK on their surface [39], making it accountable for facilitating implant of metastatic cells even in organs different than bones in several types of tumors [39–42] including RCC [43] by recruiting monocyte/macrophage cells, fundamental for pre-metastatic niche formation.

About one-third of patients with kidney cancer will develop BM during disease course with consequences in quality of life (QoL) and outcome, as their presence is a negative prognostic factor for mRCC.mRCC patients presenting RANKL-high/RANK-high/OPG-low tumors showed significantly shorter BMFS, DFS, and disease specific survival (DSS) compared to patients with other tumors, so that the expression of this molecule has been proposed as an independent prognostic factor for BMFS, DFS, and DSS [43].

Circulating Biomarkers

The term circulating biomarkers or "circulome" refers to the complex of molecules and cells released into bloodstream from healthy and pathological tissues. The interest towards these tissue-derived materials has grown progressively over the years simultaneously with the advent and improvement of liquid biopsy, a versatile, economical, and non-invasive tool which allows the evaluation of circulating tumor markers both in blood stream that in urine.

Liquid biopsy is useful for the study of some molecules of clinical interest such as circulating tumor DNA (ctDNA) and cell-free DNA (cfDNA) and circulating tumor RNA (ctRNA) and circulating noncoding RNAs (ncRNAs) which comprises microRNAs (miRNAs) and long noncoding RNAs (lnRNAs) that can be found in blood flow bounded to lipoproteins or carried by vesicular transporters, exosomes, and ectosomes [44].ctDNA has been investigated as a potential biomarker for RCC recurrence and early diagnosis of metastases [45], as a tool for monitoring end predicting treatment response [46, 47] and as a substitute of tumoral tissue to profile cancer genome [48]. cfDNA concentrations seem to be related to tumor progression; indeed, patients with progressive disease tend to have higher plasma levels of cfDNA than patients with stable disease or remission, making it a possible useful tool for predicting ccRCC course and

eventual progression; in this regard, further investigations are needed to clarify its use in clinical practice [49].miRNAs are short noncoding RNA, sequences of wide clinical interest; these small molecules take part in many biological events such as cellular communication and tumorigenesis [50–53], as previously said are often found associated with exosomes and can be secreted by cancerous cells during progression [54]. In particular, expression levels of exosomal miR-210 in RCC patients' serum have been evaluated in many recent studies; this molecule has been found to be overexpressed in ccRCC independently of clinical staging but was significantly lower in patients who have undergone resection of the primary tumor and in patients with localized disease than in those with metastatic disease [55].

Clinical Biomarker

Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health as per World Health Organization (WHO). Different parameters define obesity; the most used is body mass index (BMI) which however has several limitations; the most important one is that BMI does not consider the difference in distribution of fat tissue (subcutaneous, visceral) among different individuals. Despite obesity cannot properly be considered a biomarker, an enrichment of genes involved in angiogenesis and adipocyte hypertrophy has been observed in peritumoral fat. This status seems to lead to the formation of hypoxic regions which consequent increase of angiogenesis and inflammatory pathway expression. These data might explain the enhanced response to either VEGF-TKI targeted therapy and with ICIs in this specific population [56].

Considering these biological and molecular differences, the integration of the clinical parameter regarding nutritional status along with molecular features could be significant in improving either prognostic stratification or treatment selection.

Conclusions

The management of RCC is rapidly changing; novel therapeutic options are meant to improve patients' survival and QoL, but due to contradictory results in clinical trials and for the absence of homogeneous response across patients' subgroups, it still remains an interesting research field.

As reported, several biomarkers are emerging as a promising tool to reveal tumor heterogeneity and to understand the mechanism underling tumor response to treatment and toxicities.

Combining tumor-based biomarkers (gene expression profile) and blood-based biomarkers (ctDNA, cytokines) could be useful to acquire information regarding RCC and might be useful in the decision-making process. Nevertheless, the clinical applicability remains difficult and relegated in research context.

The advance in continuing search for biomarkers will help clinicians to reach the goal of a patient-centered approach.

Declarations

Conflict of Interest Dr. Procopio reported personal fees from AstraZeneca, Bayer, BMS, Ipsen, Janssen, MSD, Novartis, Pfizer, and Eisai outside the submitted work. Dr. Giannatempo reported personal fees from Merck and Janssen and grants from AstraZeneca and Ipsen outside the submitted work. Dr. Verzoni reported personal fees from Janssen, Ipsen, MSD, Pfizer, Merck, and Novartis outside the submitted work. The authors report no other conflicts of interest in this work. Other authors have no relevant financial or other relationship to disclose.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA A Cancer J Clin. 2020;70:7–30. https://doi.org/10.3322/caac.21590.
2. Dabestani S, Thorstenson A, Lindblad P, Harmenberg U, Ljungberg B, Lundstam S. Renal cell carcinoma recurrences and metastases in primary non-metastatic patients: a population-based study. World J Urol. 2016;34:1081–6. https://doi.org/10.1007/s00345-016-1773-y.
3. Heng DY, Xie W, Regan MM, Harshman LC, Bjarnason GA, Vaishampayan UN, et al. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. Lancet Oncol. 2013;14:141–8. https://doi.org/10.1016/S1470-2045(12)70559-4.
4. Biomarkers and surrogate endpoints. preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69:89–95. https://doi.org/10.1067/mcp.2001.113989.
5. Bennett P. Biomarkers: more of a challenge for bioanalysis than expected. Bioanalysis. 2016;8:2295–8. https://doi.org/10.4155/bio-2016-4991.
6. Nilsson R, Björkegren J, Tegnér J. On reliable discovery of molecular signatures. BMC Bioinformatics. 2009;10:38. https://doi.org/10.1186/1471-2105-10-38.
7.•• McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med. 2018;24:749–57. https://doi.org/10.1038/s41591-018-0053-3. **McDermott et al. in their exploratory biomarker analyses of phase II trial IMmotion150 reported a correlation between molecular features, patients risk categories and treatment**

responses suggesting their possible clinical utility in improving patient stratification and treatment selection.

8. Motzer RJ, Banchereau R, Hamidi H, Powles T, McDermott D, Atkins MB, et al. Molecular subsets in renal cancer determine outcome to checkpoint and angiogenesis blockade. Cancer Cell. 2020;38:803-817.e4. https://doi.org/10.1016/j.ccell.2020.10.011.
9. Rini BI, Powles T, Atkins MB, Escudier B, McDermott DF, Suarez C, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. The Lancet. 2019;393:2404–15. https://doi.org/10.1016/S0140-6736(19)30723-8.
10. Roumenina LT, Daugan MV, Petitprez F, Sautès-Fridman C, Fridman WH. Context dependent roles of complement in cancer. Nat Rev Cancer. 2019;19:698–715. https://doi.org/10.1038/s41568-019-0210-0.
11. Daugan MV, Revel M, Russick J, Dragon-Durey M-A, Gaboriaud C, Robe-Rybkine T, et al. Complement C1s and C4d as prognostic biomarkers in renal cancer: emergence of noncanonical functions of C1s. Cancer Immunol Res. 2021;9:891–908. https://doi.org/10.1158/2326-6066.CIR-20-0532.
12. •• Motzer RJ, Choueiri TK, McDermott DF, Powles T, Vano Y-A, Gupta S, et al. Biomarker analysis from CheckMate 214 nivolumab plus ipilimumab versus sunitinib in renal cell carcinoma. J Immunother Cancer. 2022;10:e004316. https://doi.org/10.1136/jitc-2021-004316. **Motzer et al. evaluated biomarkers from major pivotal trials finding a relationship between molecular characteristic and tumor response to treatment, providing some interesting considerations about biomarker-driven precision treatments development.**
13. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019;19:307–25. https://doi.org/10.1038/s41568-019-0144-6.
14. Meylan M, Sun C-M, Elaidi R-T, Moreira M, Bougouin A, Verkarre V, et al. 1451MO In-situ immune markers predict nivolumab (N) +/-ipilimumab (I) efficacy in frontline metastatic clear cell renal cell carcinoma (mccRCC): key ancillary analyses from the BIONIKK randomized trial. Ann Oncol. 2022;33:S1207. https://doi.org/10.1016/j.annonc.2022.07.1554.
15. Vano Y-A, Elaidi R, Bennamoun M, Chevreau C, Borchiellini D, Pannier D, et al. Nivolumab, nivolumab–ipilimumab, and VEGFR-tyrosine kinase inhibitors as first-line treatment for metastatic clear-cell renal cell carcinoma (BIONIKK): a biomarker-driven, open-label, non-comparative, randomised, phase 2 trial. Lancet Oncol. 2022;23:612–24. https://doi.org/10.1016/S1470-2045(22)00128-0.
16. Sato Y, Yoshizato T, Shiraishi Y, Maekawa S, Okuno Y, Kamura T, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet. 2013;45:860–7. https://doi.org/10.1038/ng.2699.
17. Gallan AJ, Parilla M, Segal J, Ritterhouse L, Antic T. BAP1-mutated clear cell renal cell carcinoma. Am J Clin Pathol. 2021;155:718–28. https://doi.org/10.1093/ajcp/aqaa176.
18. Hodges C, Kirkland JG, Crabtree GR. The many roles of BAF (mSWI/SNF) and PBAF complexes in cancer. Cold Spring Harb Perspect Med. 2016;6:a026930. https://doi.org/10.1101/cshperspect.a026930.
19. Ricketts CJ, De Cubas AA, Fan H, Smith CC, Lang M, Reznik E, et al. The Cancer Genome Atlas comprehensive molecular characterization of renal cell carcinoma. Cell Rep. 2018;23:313-326.e5. https://doi.org/10.1016/j.celrep.2018.03.075.
20. Dizman N, Philip EJ, Pal SK. Genomic profiling in renal cell carcinoma. Nat Rev Nephrol. 2020;16:435–51. https://doi.org/10.1038/s41581-020-0301-x.
21. Voss MH, Reising A, Cheng Y, Patel P, Marker M, Kuo F, et al. Genomically annotated risk model for advanced renal-cell carcinoma: a retrospective cohort study. Lancet Oncol. 2018;19:1688–98. https://doi.org/10.1016/S1470-2045(18)30648-X.
22. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43–9. https://doi.org/10.1038/nature12222.
23. Shen M, Chen G, Xie Q, Li X, Xu H, Wang H, et al. Association between PD-L1 expression and the prognosis and clinicopathologic features of renal cell carcinoma: a systematic review and meta-analysis. Urol Int. 2020;104:533–41. https://doi.org/10.1159/000506296.
24. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, et al. Costimulatory B7–H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci USA. 2004;101:17174–9. https://doi.org/10.1073/pnas.0406351101.
25. Joseph RW, Millis SZ, Carballido EM, Bryant D, Gatalica Z, Reddy S, et al. PD-1 and PD-L1 expression in renal cell carcinoma with sarcomatoid differentiation. Cancer Immunol Res. 2015;3:1303–7. https://doi.org/10.1158/2326-6066.CIR-15-0150.
26. Shin S-J, Jeon YK, Kim P-J, Cho YM, Koh J, Chung DH, et al. Clinicopathologic analysis of PD-L1 and PD-L2 expression in renal cell carcinoma: association with oncogenic proteins status. Ann Surg Oncol. 2016;23:694–702. https://doi.org/10.1245/s10434-015-4903-7.
27. Choueiri TK, Motzer RJ, Rini BI, Haanen J, Campbell MT, Venugopal B, et al. Updated efficacy results from the JAVELIN Renal 101 trial: first-line avelumab plus axitinib versus sunitinib in patients with advanced renal cell carcinoma. Ann Oncol. 2020;31:1030–9. https://doi.org/10.1016/j.annonc.2020.04.010.
28. Jilaveanu LB, Shuch B, Zito CR, Parisi F, Barr M, Kluger Y, et al. PD-L1 expression in clear cell renal cell carcinoma: an analysis of nephrectomy and sites of metastases. J Cancer. 2014;5:166–72. https://doi.org/10.7150/jca.8167.
29. Möller K, Fraune C, Blessin NC, Lennartz M, Kluth M, Hube-Magg C, et al. Tumor cell PD-L1 expression is a strong predictor of unfavorable prognosis in immune checkpoint therapy-naive clear cell renal cell cancer. Int Urol Nephrol. 2021;53:2493–503. https://doi.org/10.1007/s11255-021-02841-7.
30. Australian Pancreatic Cancer Genome Initiative, ICGC Breast Cancer Consortium, ICGC MMML-Seq Consortium, ICGC PedBrain, Alexandrov LB, Nik-Zainal S, et al. Signatures of mutational processes in human cancer. Nature. 2013;500: 415–421. https://doi.org/10.1038/nature12477
31. de Velasco G, Miao D, Voss MH, Hakimi AA, Hsieh JJ, Tannir NM, et al. Tumor mutational load and immune parameters across metastatic renal cell carcinoma risk groups. Cancer Immunol Res. 2016;4:820–2. https://doi.org/10.1158/2326-6066.CIR-16-0110.
32. Aggen DH, Drake CG, Rini BI. Targeting PD-1 or PD-L1 in metastatic kidney cancer: combination therapy in the first-line setting. Clin Cancer Res. 2020;26:2087–95. https://doi.org/10.1158/1078-0432.CCR-19-3323.
33. Yakirevich E, Patel NR. Tumor mutational burden and immune signatures interplay in renal cell carcinoma. Ann Transl Med. 2020;8:269–269. https://doi.org/10.21037/atm.2020.02.81.
34. Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. Lancet Oncol. 2017;18:1009–21. https://doi.org/10.1016/S1470-2045(17)30516-8.
35. Masini C, Iotti C, De Giorgi U, Bellia RS, Buti S, Salaroli F, et al. Nivolumab in combination with stereotactic body

radiotherapy in pretreated patients with metastatic renal cell carcinoma Results of the Phase II NIVES Study. European Urol. 2022;81:274–82. https://doi.org/10.1016/j.eururo.2021.09.016.
36. Sepe P, Martinetti A, Mennitto A, Verzoni E, Claps M, Raimondi A, et al. Prospective translational study investigating molecular predictors of resistance to first-line pazopanib in metastatic renal cell carcinoma (PIPELINE study). Am J Clin Oncol. 2020;43:621–7. https://doi.org/10.1097/COC.0000000000000719.
37. Simonetti S, Iuliani M, Stellato M, Cavaliere S, Vincenzi B, Tonini G, et al. Extensive plasma proteomic profiling revealed receptor activator of nuclear factor kappa-B ligand (RANKL) as emerging biomarker of nivolumab clinical benefit in patients with metastatic renal cell carcinoma. J Immunother Cancer. 2022;10:e005136. https://doi.org/10.1136/jitc-2022-005136.
38. Chawla S, Henshaw R, Seeger L, Choy E, Blay J-Y, Ferrari S, et al. Safety and efficacy of denosumab for adults and skeletally mature adolescents with giant cell tumour of bone: interim analysis of an open-label, parallel-group, phase 2 study. Lancet Oncol. 2013;14:901–8. https://doi.org/10.1016/S1470-2045(13)70277-8.
39. Mori K, Le Goff B, Charrier C, Battaglia S, Heymann D, Rédini F. DU145 human prostate cancer cells express functional receptor activator of NFκB: new insights in the prostate cancer bone metastasis process. Bone. 2007;40:981–90. https://doi.org/10.1016/j.bone.2006.11.006.
40. Van Poznak C. Expression of osteoprotegerin (OPG), TNF related apoptosis inducing ligand (TRAIL), and receptor activator of nuclear factor B ligand (RANKL) in human breast tumours. J Clin Pathol. 2006;59:56–63. https://doi.org/10.1136/jcp.2005.026534.
41. Chen L-M, Kuo C-H, Lai T-Y, Lin Y-M, Su C-C, Hsu H-H, et al. RANKL increases migration of human lung cancer cells through intercellular adhesion molecule-1 up-regulation. J Cell Biochem. 2011;112:933–41. https://doi.org/10.1002/jcb.23009.
42. Mikami S, Katsube K, Oya M, Ishida M, Kosaka T, Mizuno R, et al. Increased RANKL expression is related to tumour migration and metastasis of renal cell carcinomas: expression of RANKL in renal cell carcinomas. J Pathol. 2009;218:530–9. https://doi.org/10.1002/path.2567.
43. Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, et al. Regulation of cancer cell migration and bone metastasis by RANKL. Nature. 2006;440:692–6. https://doi.org/10.1038/nature04524.
44. Barth DA, Drula R, Ott L, Fabris L, Slaby O, Calin GA, et al. Circulating non-coding RNAs in renal cell carcinoma—pathogenesis and potential implications as clinical biomarkers. Front Cell Dev Biol. 2020;8:828. https://doi.org/10.3389/fcell.2020.00828.
45. Bergerot PG, Hahn AW, Bergerot CD, Jones J, Pal SK. The role of circulating tumor DNA in renal cell carcinoma. Curr Treat Options in Oncol. 2018;19:10. https://doi.org/10.1007/s11864-018-0530-4.
46. Green EA, Li R, Albiges L, Choueiri TK, Freedman M, Pal S, et al. Clinical utility of cell-free and circulating tumor DNA in kidney and bladder cancer: a critical review of current literature. European Urology Oncology. 2021;4:893–903. https://doi.org/10.1016/j.euo.2021.04.005.
47. Yamamoto Y, Uemura M, Fujita M, Maejima K, Koh Y, Matsushita M, et al. Clinical significance of the mutational landscape and fragmentation of circulating tumor DNA in renal cell carcinoma. Cancer Sci. 2019;110:617–28. https://doi.org/10.1111/cas.13906.
48. Lakshminarayanan H, Rutishauser D, Schraml P, Moch H, Bolck HA. Liquid biopsies in renal cell carcinoma—recent advances and promising new technologies for the early detection of metastatic disease. Front Oncol. 2020;10:582843. https://doi.org/10.3389/fonc.2020.582843.
49. Wan J, Zhu L, Jiang Z, Cheng K. Monitoring of plasma cell-free DNA in predicting postoperative recurrence of clear cell renal cell carcinoma. Urol Int. 2013;91:273–8. https://doi.org/10.1159/000351409.
50. Turchinovich A, Weiz L, Burwinkel B. Extracellular miRNAs: the mystery of their origin and function. Trends Biochem Sci. 2012;37:460–5. https://doi.org/10.1016/j.tibs.2012.08.003.
51. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9. https://doi.org/10.1038/ncb1596.
52. Szabó Z, Szegedi K, Gombos K, Mahua C, Flaskó T, Harda K, et al. Expression of miRNA-21 and miRNA-221 in clear cell renal cell carcinoma (ccRCC) and their possible role in the development of ccRCC. Urol Oncol: Seminars Orig Investigations. 2016;34(533):e21-533.e27. https://doi.org/10.1016/j.urolonc.2016.06.011.
53. Xu D, Dong P, Xiong Y, Yue J, Ihira K, Konno Y, et al. MicroRNA-361: a multifaceted player regulating tumor aggressiveness and tumor microenvironment formation. Cancers. 2019;11:1130. https://doi.org/10.3390/cancers11081130.
54. Greening DW, Gopal SK, Mathias RA, Liu L, Sheng J, Zhu H-J, et al. Emerging roles of exosomes during epithelial–mesenchymal transition and cancer progression. Semin Cell Dev Biol. 2015;40:60–71. https://doi.org/10.1016/j.semcdb.2015.02.008.
55. Zhang W, Ni M, Su Y, Wang H, Zhu S, Zhao A, et al. MicroRNAs in serum exosomes as potential biomarkers in clear-cell renal cell carcinoma. Eur Urol Focus. 2018;4:412–9. https://doi.org/10.1016/j.euf.2016.09.007.
56. Sanchez A, Furberg H, Kuo F, Vuong L, Ged Y, Patil S, et al. Transcriptomic signatures related to the obesity paradox in patients with clear cell renal cell carcinoma: a cohort study. Lancet Oncol. 2020;21:283–93. https://doi.org/10.1016/S1470-2045(19)30797-1.