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Immune checkpoint inhibitors in renal cell carcinoma

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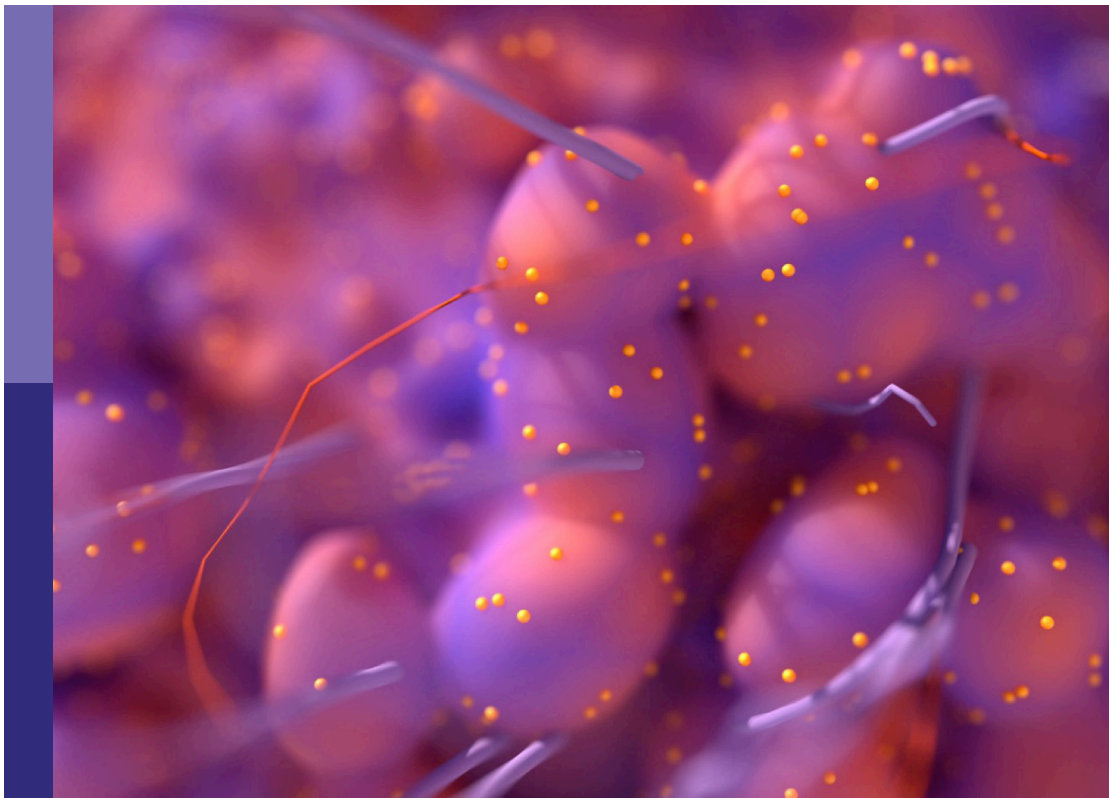

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Immune checkpoint inhibitors in renal cell carcinoma

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Table of contents

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Editorial: Immune checkpoint inhibitors in renal cell carcinoma

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KEYWORDS

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Editorial on the Research Topic
Immune checkpoint inhibitors in renal cell carcinoma

Renal cell carcinoma (RCC) has long been viewed as a tumor with unique sensitivity to immunotherapies. The clinical development of systemic cytokines interferon-alpha or interleukin-2 as front-line therapy dating to the late 1980's represented a unique treatment paradigm for metastatic carcinoma in a field dominated by use of cytotoxic chemotherapies. In the mid 2000's, molecularly targeted therapies blocking vascular endothelial growth factor receptor or mammalian/mechanistic target of rapamycin signaling were rapidly embraced for front-line management of advanced disease for their higher response and disease control rates. However, the uniform development of resistant disease proved to be a significant limitation to this approach. The recent emergence of immune checkpoint inhibitors (ICIs) blocking PD1/PDL1 or CTLA4 signaling pathways has re-established systemic immunotherapy as central to the medical management of advanced RCC. A series of positive phase III trials of ICIs or ICI/tyrosine kinase inhibitor (TKI) doublet combinations, all showing clinical benefit including superior overall survival versus sunitinib monotherapy, have established the current treatment paradigm for advanced RCC (1). Thus, practitioners are now faced with selecting their preferred treatment from among four ICI-containing doublet regimens.

The widespread adoption of ICIs in cancer therapy has encouraged detailed analysis of their unique properties. In assessing efficacy, this drug class has been associated with early response heterogeneity with some patients showing a pattern of radiographic progression that precedes a subsequent response, often described as pseudoprogression (2). In addition, durability of tumor responses even after drug discontinuation, identified as treatment free survival (TFS), is an RCC phenotype more commonly associated with ICIs than with targeted therapies (3). However, despite the proportionally better success for ICIs or ICI/TKI combination regimens vs TKIs alone, outcomes remain imperfect with most patients ultimately experiencing treatment resistance and tumor progression, or drug intolerance. Biomarkers for ICI treatment efficacy are of great interest for RCC to guide optimal selection of available treatment options. ICIs have also been associated with a unique

toxicity pattern versus other drug classes that reflects immune dysregulation and autoimmune pathology targeting normal tissues.

In the series of manuscripts responding to the Research Topic "Immune Checkpoint Inhibitors in Renal Cell Carcinoma", the contributing authors address many key issues facing clinicians and researchers who treat RCC with ICIs and who study the immunobiology of this disease. Efficacy outcomes for advanced RCC treated by front-line ICI containing doublets are summarized by Tung and Sahu who provide a comprehensive review of the current therapy landscape and introduce ongoing clinical research investigating ICIs in combination with novel agents. Jo et al. present a single center retrospective study highlighting the association of International Metastatic renal cell carcinoma Database Consortium (IMDC) risk category with the proportional benefit of ICIs versus targeted therapy. They compare real world outcomes for IMDC poor risk RCC showing better efficacy endpoints with ICI-based combinations versus TKI monotherapies. Rebuzzi et al. utilizing data from the Meet-URO-15 multicenter retrospective study of nivolumab treated RCC patients, analyzed serial blood counts from clinical safety laboratory data to assess the associations of absolute cell counts and inflammatory ratios with clinical outcomes. On-treatment neutrophil increase and increasing neutrophil to lymphocyte ratio (NLR) were negatively associated with progression free survival (PFS) and overall survival (OS) representing dynamic prognostic factors with potential clinical utility for on-treatment decision making. Bimbatti et al. addressed TFS associated with ICIs reporting on a single institution cohort of 14 RCC patients who discontinued nivolumab in the absence of disease progression. Median PFS from the date of discontinuation was 19.8 months with treatment duration > 12 months and objective response associated with longer PFS. In addition, 3 patients were re-treated with nivolumab for disease progression, and all achieved subsequent disease stability.

Based on the current treatment paradigm for advanced RCC, most patients are treated with an ICI-based regimen in the front-line setting. The role and potential benefit for ICIs as salvage therapy for patient's refractory to PD1 and/or CTLA4 blockade has not been well established in the context of prospectively enrolled, randomized, comparison clinical trials. Papathanassiou et al. conducted a systematic review compiling 10 studies totaling 500 RCC patients with ICI refractory disease who were treated with ICI-containing therapies in the second line or beyond. Aggregate efficacy outcomes showed an objective response rate (ORR) of 19% and PFS of 5.6 months with ≥ grade 3 adverse events seen in 25% of patients indicating modest efficacy and tolerable toxicity in this clinical context.

The discovery of predictive biomarkers for ICI mediated control of RCC has been an elusive target that encourages ongoing evaluation. Kim et al. report on the use of multiplexed immunohistochemistry to detect immune cell subsets in the tumor microenvironment from 24 RCC patients treated by nivolumab plus ipilimumab. Higher densities of $Foxp3^{-}CD4^{+}$ helper T cells, $CD68^{+}CD206^{-}$ M1 macrophages, and $CD137^{+}CD8^{+}$ cytotoxic T cells were associated with better PFS, with the $Foxp3^{-}CD4^{+}$ helper T cell association remaining significant in multivariate modeling. Yuan et al. report the development of a 13 gene signature for cuproptosis categorizing patients into high versus low-risk groups according to the median score. The low-risk phenotype was prognostic for better PFS and OS in The Cancer Genome Atlas data and associated with better PFS in patients treated with both ICI-based regimens and TKIs.

Immune related adverse events (IRAE) represent the unique toxicity profile of ICI-based treatment regimens. Many RCC patients have undergone nephrectomy surgery for management of the primary kidney tumor resulting in reduced renal functional capacity. Renal toxicities associated with ICIs are therefore of particular relevance to this population. Liu et al. address the incidence of renal adverse events (RAEs) for ICI-based regimens versus targeted or chemotherapies culled from 95 randomized controlled trials (including all cancer diagnoses) totaling > 40,000 patients. The overall incidence of ≥ grade 3 RAEs was 4.3%. Among ICI monotherapies, anti-CTLA4 had a higher risk of ≥ grade 3 renal adverse events (RAEs) than anti-PD1/PDL1. The anti-CTLA4/PD1 combination also had higher risk for RAEs than anti-PD1. Scarlotta et al. describe a case report of a patient treated for RCC with nivolumab plus ipilimumab who also had a history of diffuse large B cell lymphoma, in remission. The patient developed diffuse lymphadenopathy representing a diagnostic dilemma for this clinical presentation that could represent disease progression vs sarcoid-like autoimmunity versus infection. Diagnostic and management challenges for ICI associated toxicities are also addressed by Roberto et al. who highlight the value of a multidisciplinary approach to the management of high grade IRAEs.

Author contributions

ST drafted the manuscript. Both authors edited, reviewed and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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SYSTEMATIC REVIEW
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Comparative Risk of Renal Adverse Events in Patients Receiving Immune Checkpoint Inhibitors: A Bayesian Network Meta-Analysis

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Background: Immune checkpoint inhibitors (ICIs) have brought a paradigm shift to cancer treatment. However, little is known about the risk of renal adverse events (RAEs) of ICI-based regimens, especially ICI combination therapy.

Methods: We carried out a network meta-analysis of randomized controlled trials (RCTs) to compare the risk of RAEs between ICI-based regimens and traditional cancer therapy, including chemotherapy and targeted therapy. Subgroup analysis was conducted based on tumor types.

Results: Ninety-five eligible RCTs involving 40,552 participants were included. The overall incidence of RAEs, grade 3–5 RAEs, acute kidney injury (AKI), and grade 3–5 AKI was 4.3%, 1.2%, 1.3%, and 0.8%, respectively. Both ICI-based treatment regimens and traditional cancer therapy showed significantly higher risk of RAEs and AKI than the placebo. Among ICI monotherapy, anti-PD-1 (RR: 0.51, 95%CI: 0.29–0.91) was significantly safer than anti-CTLA-4 in terms of RAEs. Anti-CTLA-4 showed significantly higher toxicity than anti-PD-1 (RR: 0.33, 95%CI: 0.14-0.77), anti-PD-L1 (RR: 0.38, 95% CI:0.16-0.91), and anti-PD-1 plus anti-CTLA-4 (RR: 0.32, 95%CI: 0.12-0.87) in terms of grade 3-5 RAEs. The difference was not significant between ICI monotherapy and traditional cancer therapy, except that targeted therapy seemed the least toxic therapy in terms of the incidence of AKI. Anti-CTLA-4 plus anti-PD-1 were associated with higher risk of RAEs than anti-PD-1 (RR: 1.61, 95%CI: 1.02–2.56). The difference was not significant between other dual ICI regimens and ICI monotherapy in terms of RAEs and AKI. ICI plus chemotherapy showed increased risk of both RAEs and AKI compared with ICI monotherapy, chemotherapy, and targeted therapy. The overall results remained robust in the meta-regression and sensitivity analyses.

Conclusions: Among ICI monotherapy, anti-CTLA-4 appeared to be associated with increased toxicity, especially in terms of grade 3–5 RAEs. Anti-CTLA-4 plus anti-PD-1 were associated with higher risk of RAEs than anti-PD-1. However, the difference was not

significant between other dual ICI regimens and ICI monotherapy in terms of RAEs and AKI. ICIs plus chemotherapy seemed to be the most toxic treatment regimen in terms of RAEs, AKI, and grade 3–5 AKI.

Systematic Review Registration: PROSPERO, identifier CRD42020197039.

Keywords: treatment regimen, cancer, acute kidney injury, renal adverse events, immune checkpoint inhibitors

INTRODUCTION

Immune checkpoint inhibitor (ICI) therapy has unveiled a new era in cancer treatment, yielding an unprecedented and robust response in the treatment of different malignancies. These ICIs release inactive immune responses by blocking specific down-regulators of the immune response including cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) and its ligand, programmed cell death ligand 1 (PD-L1) (1). Although these regulators mediate an inhibitory effect on T cell response, they exert their biological effect *via* different mechanisms and on different sites (2). CTLA-4, expressed on the surface of T cells, slows down the CD4+ and CD8+ cells' activation by inhibiting the co-stimulatory signaling pathway within lymphoid organs (3, 4). PD-1, a protein receptor expressed by T cells, B cells, NK cells, and several other tumor-infiltrating lymphocytes, acts within peripheral tissues (5, 6). It functions by binding to its ligand PD–L1 on the antigen-presenting cells, leading to T cells exhaustion and inhibiting their capacity of activation and differentiation (7, 8). Therefore, by targeting these immune checkpoints, ICIs can reinvigorate T cell activity and augment antitumor immunity.

Since 2011, seven immune checkpoint-directed antibodies have been approved by the US Food and Drug Administration (FDA), and both ICI monotherapy and combination therapy have achieved great success in a variety of cancers (9–11). To improve patients' response, an increasing number of studies are focusing on regimens combining ICIs with traditional cancer therapies such as chemotherapy and targeted therapy. Based on Keynote-189, ICIs in combination with chemotherapy is now considered the standard–of–care for metastatic non-small cell lung cancer (NSCLC) (12). Most recently, ICIs combined with tyrosine kinase inhibitors has been approved for the treatment of renal cell carcinoma and endometrial cancer (13, 14).

The successful antitumor effects of ICIs are limited by the unique side effects termed immune-related adverse events (irAEs). Similar to autoimmune diseases, irAEs can affect multiple organ systems in the body. Dermatological complications are the most common, followed by gastrointestinal distress, hepatotoxicity, and endocrinopathies (15, 16). Renal toxicity is less common; however, it is attracting increasing attention as the use of ICIs continues to expand. The incidence of ICI–associated acute kidney injury (AKI) is estimated to range from 1.4% to 4.9%, with dual ICI regimens carrying an increased risk when compared with monotherapy with anti-CTLA-4, anti-PD-1, or anti-PD-L1 (17–20). Although a broad spectrum of renal lesions have been reported, tubulointerstitial nephritis (TIN) is recognized as the most common renal pathology (18, 20, 21).

The incidence and risks of renal adverse events (RAEs) in ICI monotherapy and dual ICI regimens are relatively well recognized; however, there is a new urgent need to understand the incidence and risks of ICIs in combination with traditional cancer therapy, including chemotherapy and targeted therapy. Thus, we conducted this network meta-analysis to explore the risk of RAEs in patients with ICI monotherapy and combination therapy.

Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; CTCAE, Common Terminology Criteria for Adverse Events; CTLA-4, cytotoxic T-lymphocyte antigen 4; DIC, deviance information criterion; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; FDA, Food and Drug Administration; ICIs, immune checkpoint inhibitors; ICI-AKI, immune checkpoint inhibitor-associated AKI; irAEs, immune-related adverse events; NSCLC, non-small cell lung cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RAEs, renal adverse events; RCTs, randomized clinical trials; RR, risk ratio; SUCRA, surface under the cumulative ranking; TIN, tubulointerstitial nephritis; TRAEs, treatment-related adverse events.

METHODS

This network meta-analysis was conducted according to a prespecified protocol and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (**Supplementary Table 1**) (22). Ethics committee approval was not required for this study design. The study was registered with PROSPERO (number: CRD42020197039).

Data Sources and Searches

A systematic search of the literature was conducted in PubMed, Embase, and the Cochrane Library (before June 1, 2020) without imposing any language restrictions. The search strategy is detailed in **Supplementary Table 2**. As the publication bias caused by unpublished data can significantly interfere with the relative efficacy of the network meta-analysis and modify the rankings, we also searched the ClinicalTrials.gov website (https://clinicaltrials.gov/) for unpublished or ongoing trials. Furthermore, we manually searched the reference lists of retrieved records and clinical trial registries to identify additional studies.

Study Selection

Studies were included if they met all of the following criteria: (a) randomized clinical trials (RCTs) of patients with cancer; (b) at least one treatment group received an FDA-approved ICI, as

monotherapy or combined with another ICI or traditional cancer therapy; and (c) reported data of RAEs in each group. When multiple publications covering the same study were identified, we included the one with the most recent and comprehensive data. Studies that failed to meet the above criteria were excluded. We also excluded reviews, meetings, conference abstracts, and case reports.

Two investigators (ZQ and KL) independently evaluated the title and abstract of retrieved reports, screened their full text for eligibility, and further assessed risk of bias. Clinical trials with results from ClinicalTrials.gov were also identified and included. Any discrepancy during the processes was resolved by discussion with a third reviewer (XX).

Data Extraction and Quality Assessment

ZQ entered data into an electronic spreadsheet (Microsoft Excel). KL independently checked the data and resolved disagreements by discussion. The primary outcome of the review was RAEs, which were defined as adverse events reported in the form of increased blood creatinine, decreased renal creatinine clearance, decreased urine output, oliguria, anuria, glomerulonephritis, TIN, nephritis, autoimmune nephritis, renal tubular acidosis, nephropathy toxic, nephrotic syndrome, glomerulosclerosis, kidney fibrosis, renal failure, acute renal failure, prerenal failure, postrenal failure, renal injury, renal impairment, and chronic kidney disease (CKD). Other outcomes were classified as grade 3–5 RAEs, AKI, and grade 3–5 AKI. AKI was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) sCr criteria and the Common Terminology Criteria for Adverse Events (CTCAE), specifically as a >0.3 mg/dL increase or a >1.5-fold rise in serum creatinine from baseline. We defined the grading of adverse events on the basis of the Common Terminology Criteria for Adverse Events (CTCAE) applied in individual clinical trials. When different doses of the same ICI regimen were used in a trial, we chose the one in line with the approval dose of the FDA (**Supplementary Table 3**). We did not distinguish between different chemotherapeutic or targeted drugs and considered them as one group in a trial. Quality was assessed independently by researchers in a blinded fashion. We assessed the sources of bias using the Cochrane Collaboration risk-of-bias tool (23).

Statistical Analysis

Conventional pairwise meta-analysis was initially performed taking into account the available head-to-head comparisons. We used risk ratio (RR) and its 95% credible intervals to estimate the risk of RAEs of different regimens. A standard random-effects model was applied because of the expected variation among various regimens to provide more conservative estimated effects. Statistical heterogeneity was assessed using the I-squared (I^2) statistic (24). The Bayesian network meta-analysis was conducted using random-effects generalized linear models based on the Markov chain Monte Carlo method (25). Each of the four chains was simultaneously run for 50,000 burn-ins and 100,000 inference iterations per chain to obtain posterior distribution. The convergence of the model was detected using the Gelman–Rubin method combined with a density plot and tract plot (26). For all outcomes, we summarized the evidence by drawing a network relation graph. The RAEs of different treatment regimens were ranked according to surface under the cumulative ranking (SUCRA) curve (27). League tables summarized all possible comparisons in the network, which indicated whether the estimated differences among different regimens were statistically significant. Model fit was assessed by calculating the deviance information criterion (DIC) as the sum of the posterior mean of the residual deviance and leverage pD. The transitivity assumption was evaluated by comparing the distribution of potential effect modifiers (mean age, sex ratio, sample size, and year) across treatment comparisons. In our analysis, global inconsistency was evaluated by the design-by-treatment interaction approach (28). To check the assumption of local consistency, the loop-specific approach and node-splitting method were used (29). We adopted the tau-squared (τ^2) test to evaluate the extent of heterogeneity for each outcome. Additionally, meta-regression analyses and sensitivity analyses were conducted to explore the sources of heterogeneity and ensure the validity and robustness of the findings. Further, to probe the rankings of all treatment regimens for the secondary outcomes, we conducted subgroup analyses based on different outcome definitions (grade 3–5 RAEs, AKI and grade 3–5 AKI) and cancer types. Publication bias was assessed by examining the potential presence of small-study effects *via* the visual inspection of comparison-adjusted funnel plots (29). Pairwise meta-analysis was conducted using Stata, version 13 (StataCorp LP), and NMA within the Bayesian framework was conducted using R software, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria), with the packages "gemtc 0.8-2" recalling JAGS (version 4.3.0) (30). $P<0.05$ was considered to indicate statistical significance.

RESULTS

Study Characteristics

The initial literature search yielded 10,580 records, of which 581 records were retrieved for detailed assessment (**Figure 1**). Finally, a total of 95 eligible RCTs involving 40,552 participants were selected in the network meta-analysis. The essential baseline characteristics of these RCTs are presented in **Supplementary Table 4**. Sixteen included trials assessed ≥3 treatment regimens, which were made by pairwise comparison in the meta-analysis. The mean age for participants ranged from 47.1 to 74 years, and the proportion of male subjects was 66% in the total population. The median number of study participants was 361. The overall incidence of RAEs, grade 3–5 RAEs, AKI, and grade 3–5 AKI was 4.3% (1,756 of 40,552 patients from 95 studies), 1.2% (473 of 40,290 patients from 90 studies), 1.3% (348 of 27,009 patients from 63 studies) and 0.8% (229 of 26,819 patients from 62 studies), respectively. **Supplementary Figure 1** shows the incidence of nephrotoxicity of different kinds of treatment regimens. The anti-PD-1 plus chemotherapy, anti-PD-1 plus anti-CTLA-4, and anti-PD-1 plus targeted therapy were associated with relatively higher rate of RAEs, grade 3–5 RAEs, AKI, and grade 3–5 AKI than other regimens.

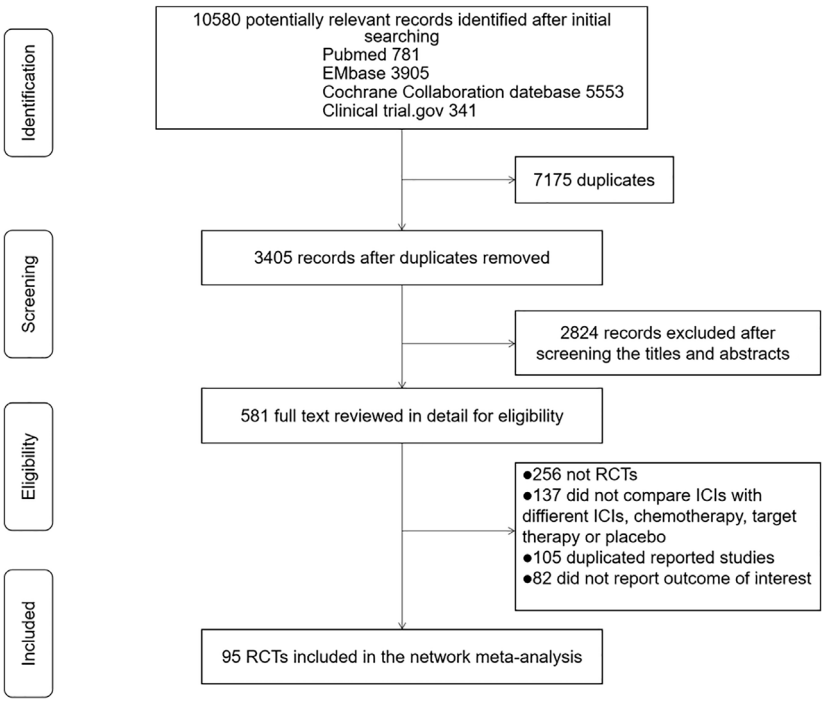

FIGURE 1 | PRISMA flow chart of literature search and selection.

Risk of Bias Assessment and Publication Bias

Overall, the quality of the trials was acceptable, with 96.8% of studies at low risk of bias for the random sequence generation, 80% at low risk of bias for allocation concealment, 95.8% at low risk of bias for incomplete outcome data, and 98.9% at low risk of bias for selective reporting. However, most of the studies were reported to have an unclear risk of bias in blinding participants and personnel (73.7%) and blinding of outcome assessment (63.2%). The risk of bias for each included trial is detailed in **Supplementary Figure 2**. In addition, inspection of comparison-adjusted funnel plots revealed no distinct asymmetry and therefore no significant risk of small-study effects was recognized (**Supplementary Figure 3**).

Conventional Pairwise Meta-Analysis

The results of the pairwise meta-analysis in terms of RAEs are shown in **Supplementary Table 5**. Anti-PD-1 plus chemotherapy (RR, 3.13; 95% CI, 2.08-4.76), anti-PD-1 plus targeted therapy (RR: 1.75, 95%CI: 1.06-2.94), anti-PD-1 plus anti-CTLA-4 (RR: 2.04, 95%CI: 1.10-3.70) and chemotherapy plus targeted therapy (RR: 2.33, 95%CI: 1.19-4.55) showed remarkably higher toxicity than anti-PD-1. Furthermore, anti-PD-1 plus chemotherapy was associated with significantly increased toxicity when compared with chemotherapy (RR: 1.99, 95%CI: 1.03-3.85) and chemotherapy plus targeted therapy (RR: 1.95, 95%CI: 1.15-3.30). The results of available direct comparisons and testing heterogeneity (I^2, τ^2, and Q) of different treatment regimens are listed in **Supplementary Table 5**. The heterogeneity was low-o-moderate despite a lack of head-to-head comparison of some treatment regimens.

Network Meta-Analysis

Figure 2 shows the network of all comparisons for RAEs. The results of the network meta-analysis in RAEs are given in **Table 1**. Moreover, we analyzed secondary outcomes to have a comprehensive understanding of the toxicity of different treatment regimens in terms of grade 3–5 RAEs, AKI, and grade 3–5 AKI. The results are shown in **Supplementary Figure 4** and **Supplementary Table 6**.

RAEs

Compared with placebo, all other treatment regimens significantly increased the risk of RAEs, with effect sizes ranging from 2.70 (95% CI: 1.33-5.82) for anti-PD-1 to 7.25 (95%CI: 3.13-17.5) for anti-PD-1 plus chemotherapy. With regard to ICI monotherapy, anti-PD-1 (RR: 0.51, 95%CI: 0.29-0.91) was significantly safer than anti-CTLA-4; however, there was no significant difference between anti-PD-1 and anti-PD-L1 with respect to safety. Anti-PD-1 plus anti-CTLA-4 (RR: 1.62, 95%CI: 1.05-2.56), anti-PD-1 plus chemotherapy (RR: 2.50, 95%CI: 1.25-0.50), and anti-PD-1 plus targeted therapy (RR: 1.75, 95%CI: 1.14-2.78) all displayed higher risk than anti-PD-1 alone. Further, anti-PD-1 plus chemotherapy (RR: 2.00, 95%CI: 1.16-3.40) and anti-PD-L1 plus chemotherapy

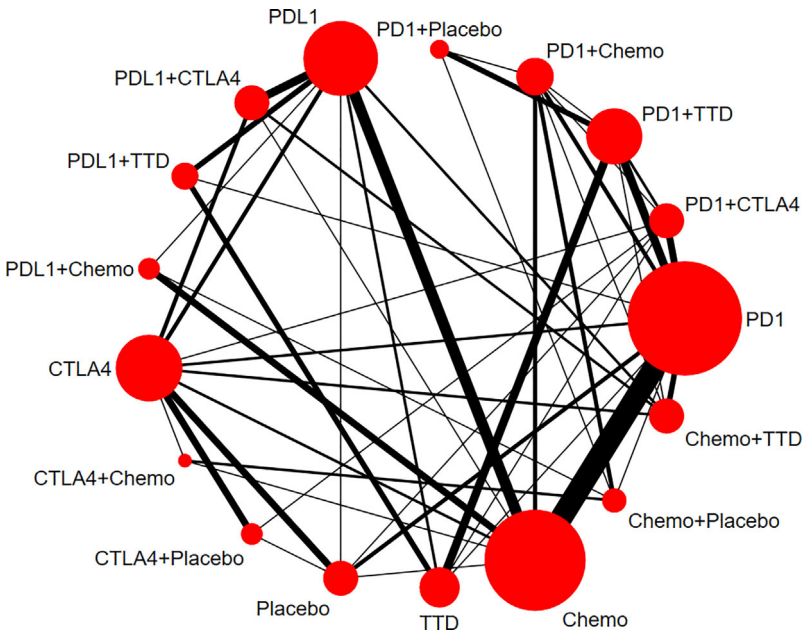

FIGURE 2 | Network plots for renal adverse events. Nodes indicate the classes which are evaluated in clinical trials. Lines represent head-to-head comparisons of the two treatment regimens indicated by the connected nodes. The thickness of lines is weighted according to the number of trials comparing the two connected treatment regimens. The size of the node is proportional to the number of trials evaluating the treatment. TTD, targeted therapy drug; Chemo, chemotherapy; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA4, cytotoxic T-lymphocyte antigen 4.

(RR: 1.85, 95%CI: 1.00-3.42) were associated with higher risk than chemotherapy. There were no significant differences between anti-PD-1 plus targeted therapy or anti-PD-L1 plus targeted therapy and targeted therapy.

Based on the ranking curves (**Figure 3** and **Supplementary Figure 5**), ICIs plus chemotherapy seemed to be the most toxic treatment regimen in terms of RAEs and had the worst rank, whereas anti-PD-1 monotherapy seemed to be the least toxic one, followed by anti-PD-L1 plus anti-CTLA-4.

Grade 3–5 RAEs

All treatment regimens enhanced the risk of grade 3–5 RAEs in varying degrees compared with placebo, except anti-PD-L1 plus anti-CTLA-4 and anti-PD-L1 plus targeted therapy. Anti-CTLA-4 showed significantly higher toxicity than anti-PD-1 (RR: 0.33, 95%CI: 0.14-0.77), anti-PD-L1 (RR: 0.38, 95%CI: 0.16-0.91), anti-PD-1 plus anti-CTLA-4 (RR: 0.32, 95%CI: 0.12-0.87), and anti-PD-L1 plus anti-CTLA-4 (RR: 0.26, 95%CI: 0.09-0.75). Furthermore, anti-PD-1 plus targeted therapy, anti-PD-1 plus chemotherapy, anti-PD-L1 plus chemotherapy, anti-CTLA-4, anti-CTLA-4 plus chemotherapy, and chemotherapy showed significantly higher risk than targeted therapy. The estimated effects were not significant between chemotherapy and ICIs plus chemotherapy.

Anti-CTLA-4 appeared to be the most toxic treatment regimen with an RR of 9.44 (95% CI: 3.66–29.7), whereas targeted therapy appeared to be the least toxic regimen with the best rank (**Supplementary Figures 5** and **7**).

Acute Kidney Injury

Apart from anti-PD-1 plus anti-CTLA-4, anti-PD-L1 plus targeted therapy, and targeted therapy, all other treatment regimens showed increased risk of AKI compared with placebo. The toxic effects of ICI monotherapy and combination therapy were not significantly different, except that anti-PD-1 plus chemotherapy had a significantly higher risk of AKI than the anti-PD-1 regimen. In addition, anti-PD-1, anti-PD-L1, anti-CTLA-4, chemotherapy, anti-PD-1 plus chemotherapy, anti-PD-1 plus targeted therapy, and chemotherapy plus targeted therapy all showed markedly higher risk of AKI than targeted therapy. ICIs plus chemotherapy seemed to be the most toxic treatment regimen, whereas targeted therapy seemed to be the least toxic one in terms of AKI (**Supplementary Figures 6** and **7**).

We had similar findings in the grade 3–5 AKI to those in AKI. Apart from anti-PD-1 plus anti-CTLA-4, anti-PD-L1 plus anti-CTLA-4, anti-PD-L1 plus targeted therapy, and targeted therapy, all other treatment regimens showed increased risk of grade 3-5 AKI compared with placebo. Moreover, all other regimens except anti-PD-L1 plus anti-CTLA-4 had significantly higher risk of grade 3-5 AKI than targeted therapy. SUCRAs and rankings were similar for AKI and grade 3–5 AKI (**Supplementary Figures 6** and **7**).

Transitivity, Inconsistency, Heterogeneity, and Sensitivity Analysis

The random consistency model had the lowest DIC value than the other three models, which manifested that it was the preferred model with a better trade-off between model fit and

TABLE 1 | Network estimates of treatment comparisons for RAEs and grade 3-5 RAEs.

PD-1	1.03 (0.53-2.02)	0.61 (0.29-1.21)	**0.54 (0.28-0.95)**	0.87 (0.47-1.62)	1.24 (0.46-3.43)	1.46 (0.68-3.41)	0.54 (0.21-1.28)	**0.33 (0.14-0.77)**	0.48 (0.17-1.27)	1.80 (0.95-3.53)	0.69 (0.40-1.13)	0.69 (0.30-1.58)	**3.10 (1.21-9.75)**
0.62 (0.39-0.98)	**PD1+CTLA4**	0.59 (0.24-1.37)	0.52 (0.21-1.19)	0.84 (0.36-1.99)	1.21 (0.38-3.88)	1.42 (0.56-3.94)	0.52 (0.17-1.51)	**0.32 (0.12-0.87)**	0.46 (0.14-1.48)	1.75 (0.78-3.99)	0.67 (0.29-1.45)	0.67 (0.24-1.86)	**3.02 (1.00-10.8)**
0.57 (0.36-0.88)	0.91 (0.54-1.55)	**PD1+TTD**	0.89 (0.37-2.05)	1.45 (0.60-3.54)	2.04 (0.66-6.87)	2.43 (0.93-6.90)	0.89 (0.30-2.64)	0.55 (0.19-1.58)	0.78 (0.24-2.59)	**2.97 (1.38-6.89)**	1.14 (0.49-2.57)	1.15 (0.41-3.14)	**5.13 (1.63-19.2)**
0.37 (0.23-0.62)	0.60 (0.32-1.14)	0.66 (0.36-1.22)	**PD1 +Chemo**	1.62 (0.77-3.58)	2.31 (0.80-7.16)	**2.72 (1.10-7.87)**	1.00 (0.38-2.65)	0.61 (0.24-1.67)	0.88 (0.32-2.56)	**3.34 (1.49-8.25)**	1.29 (0.69-2.37)	1.29 (0.53-3.25)	**5.84 (1.98-20.7)**
0.77 (0.47-1.26)	1.24 (0.67-2.32)	1.36 (0.75-2.48)	**2.08 (1.09-3.92)**	**PDL1**	1.42 (0.57-3.71)	1.68 (0.75-4.10)	0.62 (0.24-1.51)	**0.38 (0.16-0.91)**	0.55 (0.19-1.56)	2.06 (0.96-4.61)	0.80 (0.44-1.32)	0.79 (0.32-1.96)	**3.54 (1.31-12.2)**
0.91 (0.46-1.82)	1.46 (0.68-3.24)	1.60 (0.75-3.53)	**2.44 (1.11-5.41)**	1.18 (0.63-2.24)	**PDL1+CTLA4**	1.18 (0.36-4.06)	0.43 (0.12-1.42)	**0.26 (0.09-0.75)**	0.38 (0.10-1.40)	1.45 (0.46-4.53)	0.56 (0.20-1.40)	0.56 (0.20-1.66)	2.51 (0.72-10.6)
0.84 (0.43-1.61)	1.34 (0.64-2.82)	1.47 (0.73-2.96)	2.24 (0.99-4.92)	1.08 (0.54-2.12)	0.92 (0.38-2.16)	**PDL1+TTD**	0.37 (0.11-1.09)	**0.22 (0.07-0.66)**	0.32 (0.09-1.07)	1.23 (0.56-2.55)	0.47 (0.18-1.06)	0.47 (0.15-1.39)	2.12 (0.63-8.17)
0.40 (0.20-0.80)	0.64 (0.29-1.45)	0.71 (0.32-1.57)	1.08 (0.49-2.33)	0.52 (0.25-1.09)	0.44 (0.18-1.06)	0.48 (0.20-1.21)	**PDL1 +Chemo**	0.61 (0.20-1.97)	0.88 (0.27-2.95)	**3.35 (1.20-10.1)**	1.29 (0.57-2.80)	1.29 (0.41-4.03)	**5.81 (1.73-23.6)**
0.51 (0.29-0.91)	0.82 (0.43-1.58)	0.91 (0.46-1.79)	1.38 (0.67-2.79)	0.66 (0.34-1.27)	0.56 (0.26-1.19)	0.61 (0.27-1.41)	1.28 (0.54-2.96)	**CTLA4**	1.44 (0.44-4.66)	**5.48 (2.00-15.5)**	2.09 (0.85-4.96)	2.09 (0.78-5.72)	**9.44 (3.66-29.7)**
0.47 (0.19-1.17)	0.76 (0.28-2.04)	0.83 (0.31-2.23)	1.27 (0.48-3.27)	0.61 (0.23-1.61)	0.52 (0.17-1.50)	0.57 (0.19-1.71)	1.17 (0.40-3.39)	0.92 (0.35-2.42)	**CTLA4 +Chemo**	**3.81 (1.21-12.3)**	1.46 (0.53-3.73)	1.46 (0.43-4.93)	**6.57 (1.81-28.9)**
0.86 (0.54-1.38)	1.37 (0.80-2.40)	1.51 (0.97-2.39)	**2.31 (1.20-4.37)**	1.11 (0.62-1.99)	0.94 (0.43-2.02)	1.03 (0.56-1.90)	2.14 (0.96-4.74)	1.67 (0.84-3.39)	1.82 (0.67-5.00)	**TTD**	**0.38 (0.17-0.79)**	0.39 (0.14-1.04)	1.73 (0.57-6.01)
0.74 (0.52-1.06)	1.19 (0.69-2.07)	1.31 (0.78-2.23)	**2.00 (1.16-3.40)**	0.96 (0.61-1.50)	0.82 (0.42-1.55)	**0.28 (0.12-0.59)**	**1.85 (1.00-3.42)**	1.45 (0.79-2.68)	1.58 (0.64-3.95)	0.87 (0.50-1.49)	**Chemo**	1.00 (0.43-2.43)	**4.52 (1.68-15.4)**
0.48 (0.27-0.87)	0.77 (0.38-1.56)	0.85 (0.43-1.68)	1.30 (0.65-2.52)	0.63 (0.32-1.20)	0.53 (0.24-1.14)	0.58 (0.25-1.33)	1.20 (0.51-2.82)	0.94 (0.46-1.94)	1.02 (0.36-2.90)	0.56 (0.28-1.13)	0.65 (0.35-1.21)	**Chemo +TTD**	**4.52 (1.39-16.5)**
2.70 (1.33-5.82)	**4.33 (2.03-9.80)**	**4.77 (2.14-11.2)**	**7.25 (3.13-17.5)**	**3.49 (1.58-8.17)**	**2.96 (1.19-7.68)**	**3.24 (1.27-8.70)**	**6.73 (2.59-18.3)**	**5.26 (2.59-11.5)**	**5.75 (1.91-18.0)**	**3.15 (1.39-7.46)**	**3.63 (1.71-8.20)**	**5.61 (2.35-14.1)**	**Placebo**

Network estimates of treatment comparisons for RAEs (on the lower triangle) and grade 3-5 RAEs (on the upper triangle). The summary estimates are risk ratios (RRs) and 95% confidence intervals. For RAEs, the column-defining treatment is compared to the row-defining treatment, and RRs < 1 favor the column-defining treatment. For grade 3-5 RAEs, the row-defining treatment is compared to the column-defining treatment, and RRs < 1 favor the row-defining treatment. Significant results are in bold.

RAEs, renal adverse events; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA4, cytotoxic T-lymphocyte antigen 4; Chemo, Chemotherapy; TTD, Targeted therapy drug.

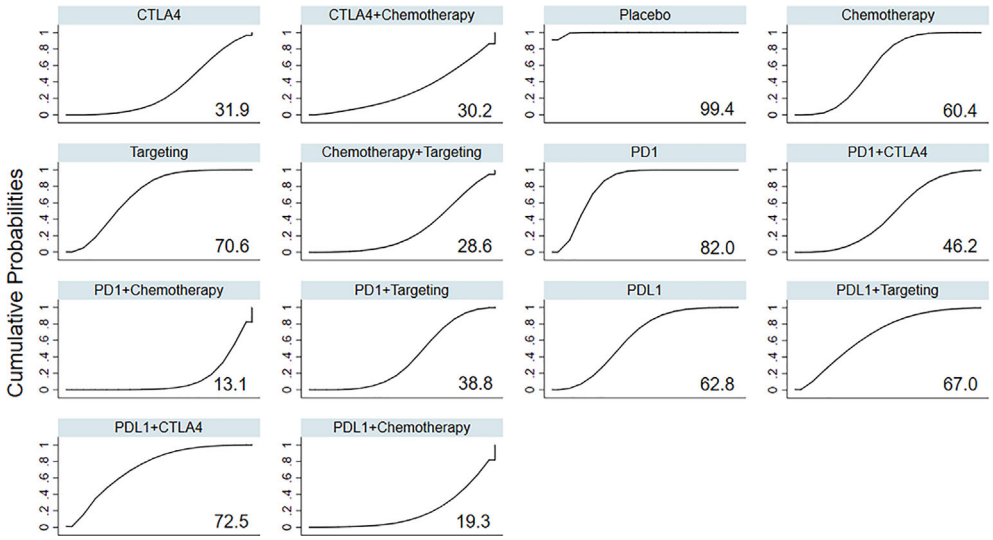

FIGURE 3 | Rankings of SUCRA for the risk of RAEs. SUCRA, surface under the cumulative ranking; RAEs, renal adverse events; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA4, cytotoxic T-lymphocyte antigen 4.

complexity (**Supplementary Table 7**). Assessment of transitivity for RAEs indicated that the median age, sex ratio, sample size, and trial start year across treatment comparisons were relatively similar and thus no threats to the transitivity assumption were identified (**Supplementary Figure 8**). The "design-by-treatment" interaction models found no evidence for global inconsistency for all outcomes. Concerning the local inconsistency, the loop-specific method (**Supplementary Figure 9**) and node-split model (**Supplementary Table 8**) revealed no significant discrepancy between the direct and indirect comparisons, except for one comparison (placebo *vs* anti-PD-L1, p=0.014). The median heterogeneity (τ^2) was estimated at 0.20 (95%CI: 0.09–0.40) for RAEs, 0.12 (95%CI: 0.00–0.57) for grade 3–5 RAEs, 0.12 (95%CI: 0.00–0.64) for AKI, and 0.17 (95%CI: 0.00–1.00) for grade 3–5 AKI, all suggesting low heterogeneity. Meta-regression analysis for RAEs revealed that tumor types might be a source of heterogeneity (**Supplementary Table 9**). Thus, we performed a subgroup analysis based on tumor types, which showed that the distribution of SUCRA values remarkably varied across different cancers in terms of RAEs (**Supplementary Table 10** and **Supplementary Figure 10**). Thus, it is reasonable to infer that tumor type may be a source of heterogeneity, and therefore our findings may not directly apply to different kinds of tumors.

It was worth noting that the effects of three kinds of regimens—anti-PD-1 plus anti-CTLA-4, anti-PD-1 plus targeted therapy, and anti-CTLA-4—were not significant when compared to anti-PD-1 in certain sensitivity analyses (**Supplementary Table 11**). Reduced sample size may be the reason for the statistically non-significant RRs and wide confidence intervals. However, there were no obvious changes in the most and least toxic treatment regimens. Hence, the overall results were relatively stable and robust.

DISCUSSION

This network meta-analysis included 95 RCTs involving 40,552 patients and compared 14 treatment regimens. In this study, we explored the RAEs in patients with ICIs, which manifest not only as AKI but also as other types of renal damage that may not meet the criteria of AKI. Both ICI-based treatment regimens and traditional cancer therapies showed significantly higher risk of RAEs than placebo. With regard to ICI monotherapy, anti-CTLA-4 showed remarkably higher risk of RAEs than anti-PD-1 and significantly greater risk of grade 3–5 RAEs than anti-PD-1 and anti-PD-L1 regimens. We did not find significant differences between ICI monotherapy and traditional cancer therapy in terms of RAEs. However, chemotherapy and ICI monotherapy both incurred significantly higher odds of AKI than targeted therapy. Anti-CTLA-4 plus anti-PD-1 were associated with higher risk of RAEs than anti-PD-1. The difference was not significant between other dual ICI regimens and ICI monotherapy in terms of RAEs and AKI. In addition, ICI plus chemotherapy showed increased risk of both RAEs and AKI to varying degrees than ICIs monotherapy, chemotherapy, and targeted therapy.

Our study found that the overall incidence of RAEs, grade 3–5 RAEs, AKI, and grade 3–5 AKI was 4.3%, 1.2%, 1.3%, and 0.8%, respectively. However, the incidence of AKI was found to be 15.5–17% in patients receiving ICIs in some retrospective studies (31–34). The main reason for this difference may be because of the different samples of patients enrolled. Unlike the general hospital populations in retrospective studies, patients in RCTs are always in a better condition, usually with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. In addition, patients in RCTs are always highly selected. For example, patients with active brain metastases, autoimmune

disease, or human immunodeficiency virus infection were excluded in some RCTs. In addition, the incidence of AKI was probably overestimated in retrospective studies as AKI due to other reasons (e.g., hemodynamic, sepsis-related, or obstructive AKI) may also be included. Whereas by including RCTs only, our study focused on ICI–related AKI. Therefore, our meta-analysis may reflect the incidence of AKI with less bias.

Our study suggested that among ICI monotherapy, anti-CTLA-4 showed remarkably higher risk of RAEs than anti-PD-1 and significantly increased risk of grade 3–5 RAEs than the anti-PD-1 and anti-PD-L1 regimens. These differences in the risk of RAEs may be attributed to the individual mechanisms of action of each medication. Although both anti-CTLA-4 and anti-PD-1 can restore antitumor immunity, they function in distinct ways. CTLA-4 exerts its regulatory effect during the early phase of the immune response within lymphoid organs. PD-1, on the other hand, exerts its regulatory effect later in the course of T cell activation within peripheral tissues (6). PD-L1, the ligand of PD-1, is expressed in the kidney tubules. Anti-PD-1 is speculated to alter T cell immune tolerance against endogenous antigens in the kidney or concomitant drugs that might trigger AIN (20, 35). The upstream and less specific effect of anti-CTLA-4 may be responsible for higher toxicity compared to anti-PD-1.

Previously, a study concluded that AKI occurred more frequently in patients who received dual ICI therapy than in patients who received ICI monotherapy (20). However, as an increasing number of ICIs are approved by the FDA in a larger sample of patients, the risk of dual ICI therapy needs to be re-evaluated. Recently, several retrospective cohort studies in different centers have found that ICI combination therapy was not a risk factor for AKI (31, 34, 36). Including the most recent studies, our study found that anti-CTLA-4 plus anti-PD-1 was associated with higher risk of RAEs than anti-PD-1. However, this difference was not significant between anti-PD-1 plus anti-CTLA-4 and anti-PD-1 in terms of AKI. More comprehensive studies and further analyses are needed to determine the incidence of RAEs of dual ICI therapies. Our study implied that ICIs plus chemotherapy is the most toxic treatment regimen in terms of RAEs, AKI, and grade 3–5 AKI. One reason for this may be the different mechanism of ICIs and chemotherapy. Conventional chemotherapeutic drugs can induce AKI by injuring multiple renal compartments including renal microvasculature, glomerulus, renal interstitium, and tubular segments (37). Drugs such as platinum-containing regimens and pemetrexed can cause direct cellular toxicity owing to their excretion through tubular cells, development of inflammation and oxidative stress, and activation of apoptotic and necrotic signaling pathways (38). Another reason may be the synergistic effects of ICIs and chemotherapy. Chemotherapy was reported to enhance the expression of PD-L1, thus improving the antitumor activity of ICIs when combining immunotherapy with chemotherapy (39, 40). In our meta-analysis, dual ICI therapy and ICIs plus targeted therapy seemed to be less toxic than ICIs plus chemotherapy. Therefore, they may be considered as a priority for patients who showed no response to ICI monotherapy or with poor kidney function.

The incidence of AKI was reported to be associated with increased mortality and morbidity and limited use of treatment regimens in patients with cancer. Severe AKI is also known to be associated with longer length of hospital stay and higher daily costs in hospital (41–43). Thus, clinicians must tailor treatment options with a better trade-off between benefits and toxicity, especially in patients with a high risk of RAEs. Our analysis suggested that anti-PD-1 seemed to be the least toxic regimen in terms of RAEs, making it a suitable choice of treatment. To our knowledge, this is the largest and most comprehensive study to compare the risk of RAEs among ICI-based therapy. Furthermore, we provide the incidence and risks of RAEs of ICIs combined with traditional cancer therapy which is still poorly understood. Our meta-analysis has some limitations. First, it was conducted at the study level rather than the individual patient data level, as potentially important variables at the patient level such as background nephropathy were not imported in the analysis. RCTs with more comprehensive data are needed to nullify these factors. Second, although the results remained stable after the meta-regression of cancer types, subgroup analysis suggested that the risk of RAEs varied remarkably across different cancer types that might be attributed to the property intrinsic to specific cancer types. The results could be misinterpreted when evaluating the possible reasons for renal impairment in such cases (disease-related *vs.* treatment-related). Input from other specialties (e.g., nephrologists and urologists) is of paramount significance in the individual management of such cases. Third, we performed analysis on different ICI classes instead of individual ICIs and particular doses, which might lead to variations in study outcomes. Similarly, different chemotherapeutic or targeted drugs with different incidences of RAEs were defined as one class that might be a source of heterogeneity. Nonetheless, differentiating treatment regimens based on individual drugs and particular dosage was not feasible due to limited samples. Finally, because patients with end stage renal disease (ESRD) are usually excluded from clinical trials, these results cannot be generalized to all patients. More data and further analysis in ESRD patients are necessary for a more in-depth understanding of the application of ICIs.

Conclusion

Our network meta-analysis has highlighted the risks of RAEs between ICI monotherapy, ICI combination therapy, and traditional cancer therapy and provided oncologists with a nephrology perspective of choosing different treatment regimens. Further studies are needed for a better understanding of RAEs among patients with different cancer types, using different ICI doses and with different kidney function.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Concept and design: KL and HM. Extraction and collection of data: ZQ, KL, and XX. Statistical analysis: KL, TL, and YG. Drafting and revision of manuscript: KL, ZQ, and HM. Supervision: HM and CX. Final approval of manuscript: KL, ZQ, XX, TL, YG, HM, and CX. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.662731/full#supplementary-material

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REVIEW
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Immune Checkpoint Inhibitor in First-Line Treatment of Metastatic Renal Cell Carcinoma: A Review of Current Evidence and Future Directions

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The incidence of renal cell carcinoma (RCC) is rising and metastatic RCC carries a very poor prognosis. The treatment paradigm for metastatic RCC has shifted dramatically in the last decade with multi-targeted tyrosine kinase inhibitors (TKI) previously used as first-line treatment but its utility is limited by short-lived efficacy and rapid disease progression. The dysregulation of immune cells in the tumour microenvironment contributes to unregulated growth of RCC. Thus, the use of immune checkpoint inhibitors has become first-line treatment for metastatic RCC and has offered dramatic improvement in clinical benefit and survival. Treatment with immune checkpoint inhibitor in combination with TKI appears to be promising in offering even greater response rates. The treatment for metastatic RCC continues to evolve and ongoing advances with new targeted agents and biomarkers are needed to continue to improve prognosis in the future.

Keywords: metastatic renal carcinoma, immune checkpoint inhibition (ICI), anti-VEGF (vascular endothelial growth factor) agents, tyrosine kinase inhibitions (TKIs) therapy, biomarker

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INTRODUCTION

Renal cell carcinoma (RCC) has an incidence of approximately 400,000 cases per year globally, which is highest in North America, Europe and Australia (1). The incidence of RCC is rising over the last 50 years, which is attributable to increasing detection on imaging and increasing exposure to risk factors including obesity and alcohol consumption, particularly in developed countries (2).

The prognosis of RCC is poor as 30% of patients have metastatic disease at diagnosis with a 5-year survival rate of only 12% (3). Clear cell RCC (ccRCC) is the most common histological subtype and accounts for over 75% of RCCs, in comparison to non-clear cell RCC (nccRCC), which consists of 15 histological subtypes, including papillary and chromophobe histology (4). Prognosis can be conferred using the International Metastatic Renal Cell Carcinoma Database (IMDC), which may be used to assess risk in individual patients and can guide treatment decisions (5). Factors included in the IMDC are anaemia, neutrophilia, thrombocytosis, hypercalcaemia, Karnofsky performance status of less than 80 and less than 1 year from diagnosis to first-line systemic therapy (5). The presence of brain, bone or liver metastasis as the first site of metastatic disease prior to treatment was identified as a newly validated prognostic factor, which was associated with worse overall survival in the groups with favourable and intermediate IMDC risk (6).

The advent of targeted treatment such as tyrosine kinase inhibitors improved survival outcomes for patients with metastatic RCC in the last decade (7). However, more recently, the use of immune checkpoint inhibitors has offered further improvement in outcomes for patients. This has dramatically altered the treatment paradigm for metastatic RCC and immune checkpoint inhibitors are increasingly used as the first-line treatment for metastatic ccRCC (**Figure 1**). This review will discuss the mechanism of immune checkpoint inhibitor in treatment of metastatic RCC, the key evidence supporting its use as first-line treatment and future research directions.

MOLECULAR PATHOGENESIS OF RCC

The development of RCC is underpinned by abnormal angiogenesis. The von Hippel-Lindau (VHL) gene is a tumour suppressor gene that regulates activity of hypoxia-induced factor (HIF) and expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (8). VHL is dysfunctional or inactivated in over 80% of ccRCC, resulting in increased HIF activity and overexpression of VEGF and PDGF which contributes to uncontrolled angiogenesis and tumour growth (8). Tyrosine kinase inhibitors (TKI) that inhibit VEGF pathway are anti-angiogenic and suppress tumour growth, with demonstrated efficacy in treatment of RCC (9). Anti-VEGF TKIs including sunitinib and pazopanib were previously used as first-line treatment of metastatic RCC. However, despite its initial efficacy, anti-tumour response is short-lived and tumour resistance inevitably develops during TKI treatment (10).

RCC is highly immunogenic and contributes to mobilisation of immune cells such as Tumour Infiltrating Lymphocytes (TILs) and natural killer cells into the tumour microenvironment, which promotes tumour growth (11, 12). Further, Programmed Death Ligand 1 (PD-L1) is widely expressed in RCC, which illustrates the importance of the PDL-1/PDL1 checkpoint in regulating tumour growth in RCC (12). Overexpression of PDL1 and its interaction with inhibitory PD-1 receptors results in downregulation and anergy of T cells, therefore downregulating host immune response against RCC (12–14). Immune checkpoint inhibitors including PD-1 inhibitors, pembrolizumab and nivolumab promote a long-lasting host immune response against tumour growth by inhibiting tumour-induced downregulation of host T cells (14).

IMMUNE CHECKPOINT INHIBITOR TREATMENT IN METASTATIC ccRCC

The use of immune checkpoint inhibitors nivolumab and ipilimumab is now approved for first-line treatment of intermediate and poor-risk metastatic RCC and has demonstrated improved overall survival across multiple clinical trials (**Table 1**). Nivolumab is a PD-1 inhibitor which blocks the interaction of PD-1 on T cells with PD-L1, thereby preventing T cell inactivation (14). Nivolumab demonstrated anti-tumour activity and efficacy in a phase II trial in second-treatment of metastatic ccRCC, that had been previously treated with an anti-angiogenic agent (24). The objective response rate of nivolumab was approximately 20% and pleasingly 40% of responders had durable responses at 24 months (24). In the phase III trial, CheckMate-025, nivolumab used in a second-line treatment setting, demonstrated a higher objective response rate of 25% compared to 5% in everolimus and a significant increase in overall survival of 25 months compared to 19.6 months in the everolimus group (25).

In the pivotal phase III trial CheckMate-214, treatment with nivolumab and ipilimumab in the first-line setting for metastatic ccRCC resulted in a higher response rate (42% *vs.* 27%, p<0.001), progression-free survival and a significant increase in 12-month overall survival rate (80% *vs.* 72%, p<0.001), when compared to the control arm of sunitinib in those with intermediate or poor IMDC risk (15). The higher response rate and overall survival benefit offered by nivolumab and ipilimumab in the groups with intermediate and poor IMDC risk was ongoing after 4 years of follow up, demonstrating a durable response, with a 4-year overall survival rate of 50% compared to 35.8% in the control arm (16). Moreover, 10% of patients achieved complete response

FIGURE 1 | Timeline of FDA-approved treatment for metastatic RCC in first-line setting.

TABLE 1 | Summary of key phase III trials in the use of immune checkpoint inhibitors in first-line treatment of metastatic RCC.

Phase III Trial	Intervention	Control	Histology	Objective response rate	Progression Free Survival	Overall Survival
CheckMate 214, 2018 (15, 16)	Nivolumab (3mg/kg) & ipilimumab 1mg/kg) followed by nivolumab 3mg/kg every 2 weeks	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell	*Fav IMDC risk:* 29.6 *vs*. 51.6% p=0.0005 *Intermediate & poor IMDC risk:* 41.9 *vs*. 26.8% p<0.0001 *ITT:* 39.1 *vs*. 32.4 p=0.0134	*Fav IMDC risk:* 12.4 *vs*. 28.9 months HR 1.84 95% CI (1.29-2.62) *Intermediate & poor IMDC risk:* 11.2 *vs*. 8.3 months HR 0.74 95% CI (0.62-0.88) *ITT:* 12.2 *vs* 12.3 months HR 0.89 95% CI (0.76-1.05)	*Fav IMDC risk:* HR 0.93 95% CI 0.62-1.4 OS not reached *Intermediate & poor IMDC risk:* 48.1 *vs*. 26.6 months 50% *vs*. 35.8% HR 0.65 95% CI (0.54-0.78) *ITT:* 46.7 *vs*. 38.4 months 53.4% *vs*. 43.3% HR 0.69 95% CI (0.59-0.81)
JAVELIN Renal 101, March 2019 (17, 18)	Avelumab (10mg/kg) & axitinib 5mg twice daily	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell	51.4% *vs*. 25.7% p value not available	13.3 *vs*. 8.0 months p<0.0001	HR 0.796 95% CI 0.616-1.027 p=0.0392 (did not reach pre-specified significance level)
KEYNOTE-426, March 2019 (19, 20)	Pembrolizumab 200mg & axitinib 5mg twice daily	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell	59.3% *vs*. 35.7% p< 0.001	15.4 *vs*. 11.1 months p<0.0001	HR 0.68 95% CI 0.55-0.85 p=0.0003 Median OS not reached
IMmotion151, May 2019 (21)	Atezolizumab 1200mg & bevacizumab 15mg/kg	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell Sarcomatoid allowed	43% *vs*. 25% p value not available	11.2 *vs*. 8.4 months p=0.0219	63% *vs*. 60% at 24 months p=0.4751
CheckMate-9ER 2020 (22)	Nivolumab 240mg & cabozantinib 40mg daily	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell Sarcomatoid allowed	55.7% *vs*. 27.1% p<0.0001	16.6 *vs*. 8.3 months HR 0.51 95% CI 0.41-0.64 p<0.0001	85.7% *vs*. 75.6% at 12 months HR 0.6, 98% CI 0.4-0.89 P=0.001
CLEAR, 2021 (23)	*Arm A:* lenvatinib & pembrolizumab *Arm B:* lenvatinib & everolimus	Sunitinib 50mg daily for 4 weeks on, 2 weeks off	Clear cell Sarcomatoid allowed	71% *vs*. 53.5% *vs*. 36.1% p value not available	*Arm A* vs. *control:* 23.9 *vs*. 9.2 months p<0.001 *Arm B* vs. *control:* 14.7 *vs*. 9.2 months p <0.01	*Arm A* vs. *control:* HR 0.66 95% CI 0.49-0.88 p=0.005 OS not reached *Arm B* vs. *control:* HR 1.15 95% CI 0.88-1.5 p=0.3 OS not reached

in the intervention arm across all IMDC risk groups, whereas treatment with sunitinib only offered a complete response rate of 1.4% and 6.5% in the intermediate to poor risk and favourable risk groups respectively (16). However, PD-L1 expression did not predict treatment response and survival benefit was observed independent of PD-L1 expression. Despite the use of two immune checkpoint inhibitors, there was a lower incidence of grade 3 and 4 treatment-related toxicities observed in the intervention arm in comparison to the use of sunitinib. Toxicities from nivolumab and ipilimumab were similar to that observed in immune checkpoint inhibitor studies in other solid organ malignancies, the most common of which included fatigue, pruritus, diarrhoea, rash and nausea. However, the incidence of grade 3 or above toxicities was still high at 46%. High dose corticosteroid treatment was required in 36% of patients experiencing toxicities, higher than when compared to treatment with immune checkpoint inhibitor combined with an anti-VEGF agent. Nonetheless, patient-reported quality of life was higher in the those who received immune checkpoint inhibitor treatment compared to sunitinib. This trial was practice-changing as immune checkpoint inhibitor with nivolumab and ipilimumab became the new standard-of-care first-line treatment for intermediate or poor risk metastatic RCC and was approved by the FDA in April 2018 for this indication.

More recently, Keynote-427, a phase II study investigated the efficacy of single-agent pembrolizumab in treatment-naïve

metastatic RCC. Cohort A of this study recruited patients with ccRCC and results demonstrated efficacy of pembrolizumab with an objective response rate of 36.4%, progression free and overall survival rates of 22.3% and 70.8% respectively at 24 months of follow up. This benefit was observed regardless of PD-L1 expression and IMDC risk. The incidence of grade 3 or above toxicity was 30%, the most common of which was colitis. High dose corticosteroid treatment was required in 44% of cases of immune-related toxicities. Pembrolizumab may be a possible treatment option to TKI in those with favourable-risk disease with manageable toxicities. However, this study is limited by its single-arm design, therefore a phase III trial would be required to compare its efficacy and safety with sunitinib or ipilimumab with nivolumab (26).

IMMUNE CHECKPOINT INHIBITOR & ANTI-VEGF TREATMENT IN METASTATIC ccRCC

More recently, there is emerging evidence to support the use of immune checkpoint inhibitor in combination with anti-VEGF targeted agents for treatment of metastatic RCC in the first-line setting (**Table 1**). Anti-VEGF agents are important in their role in anti-angiogenesis, it is hypothesised that these agents are also important in moderating the immune system by promoting trafficking of immune cells to tumour microenvironment (27). Therefore, it is proposed that the combination of immune checkpoint inhibitor with anti-VEGF agents would act synergistically in reducing tumour burden.

The phase III trial JAVELIN Renal 101 demonstrated efficacy of PD-L1 inhibitor avelumab in combination with anti-VEGF agent axitinib in treatment-naive metastatic ccRCC (17). Treatment with avelumab and axitinib was associated with a higher response rate (51.4% *vs.* 25.7%) and a significantly higher progression-free survival (13.3 *vs.* 8 months, p< 0.0001) in comparison to the control arm of sunitinib. This benefit was observed regardless of PD-L1 level and IMDC risk. However, avelumab and axitinib did not offer a significant overall survival benefit compared to the control arm in an updated analysis in 2020 (18). Common toxicities associated with avelumab and axitinib include hypertension and skin toxicity but hepatotoxicity was more prevalent in the sunitinib group. Nonetheless, the FDA approved the use of avelumab and axitinib as first-line treatment for metastatic RCC in 2019.

The phase III trial, Keynote-426 delivered promising results for the use of PD-1 inhibitor pembrolizumab and anti-VEGF agent axitinib in first-line treatment for metastatic ccRCC (19, 20, 28). Treatment with pembrolizumab and axitinib demonstrated a significantly higher objective response rate (59.3% *vs.* 35.7%, p< 0.001), progression-free survival (15.4 *vs.* 11.1 months, p<0.0001) and overall survival (HR 0.68, 95% CI [0.55-0.85] p=0.0003) in comparison to sunitinib. This benefit was observed regardless of PD-L1 expression or IMDC risk. There were no unexpected treatment toxicities but there was a higher incidence of hepatotoxicity and rates of treatment discontinuation in the intervention arm. Hypertension and diarrhoea were common toxicities in both groups. Treatment with pembrolizumab and axitinib appears to offer durable anti-tumour response at long-term follow up with an objective response rate of 85%, progression-free survival and overall survival rates of 94.7% and 74.8% at 36 months respectively (28). Results from Keynote-426 are practice-changing as the combination of pembrolizumab and axitinib was approved by the FDA in April 2019 for first-line treatment of metastatic RCC.

The phase III trial, IMmotion151 included patients with ccRCC with sarcomatoid differentiation, which accounted for 16% of the study population. In this trial, first-line treatment with PD-L1 inhibitor atezolizumab and anti-VEGF monoclonal antibody bevacizumab was associated with a higher response rate (43% *vs.* 25%) and significant improvement in progression-free survival (11.2 *vs.* 8.4 months, p=0.02) compared to sunitinib but this did not translate into an overall survival benefit (21). Treatment with atezolizumab and bevacizumab was well tolerated with a lower incidence of grade 3 or more toxicities and rates of treatment discontinuation compared to sunitinib. Immune-related toxicities from immune checkpoint inhibitor were as expected, however with the addition of bevacizumab-related toxicities including hypertension and proteinuria. Despite a benefit in overall survival was not observed in this trial, this treatment appears to have activity in the group of ccRCC with sarcomatoid differentiation on subgroup analyses. This treatment regimen has not been granted FDA approval.

The phase III trial, Checkmate-9ER included patients with ccRCC with sarcomatoid differentiation, which constituted 11.5% of the study population and investigated the role of nivolumab and cabozantinib, a second-generation anti-VEGF agent, in treatment-naïve advanced ccRCC (22). Results from this trial are encouraging, treatment with nivolumab and cabozantinib was associated with significantly higher response rate (55.7% *vs.* 27.1%, p<0.0001), longer progression-free survival (16.6 *vs.* 8.3 months, p<0.0001) and 12-month overall survival (85.7% *vs.* 75.6%, p=0.001) compared to sunitinib. This benefit was observed across all subgroups including the group with ccRCC with sarcomatoid differentiation. Survival benefit was observed independent of PD-L1 expression and IMDC risk. No unexpected treatment-related adverse events were identified although rates of hepatotoxicity were higher in the intervention group. 19% of patients in the intervention arm required high dose corticosteroid treatment due to immune-related toxicities. However, patient-reported quality of life was greater in the intervention arm compared to sunitinib. First-line treatment with nivolumab and cabozantinib for metastatic RCC was most recently FDA-approved in January 2021 based on results from this trial. However, follow-up duration in this trial is reasonably short at 18 months and therefore durability of treatment response will need to be assessed at long-term follow up.

The phase III trial, CLEAR has recently been completed and investigated the efficacy of anti-VEGFR TKI lenvatinib either in combination with everolimus alone or combined with both everolimus and pembrolizumab in treatment-naïve ccRCC (23). This trial also included patients with ccRCC with sarcomatoid differentiation, which constituted approximately

20% of the study population. Treatment with lenvatinib and pembrolizumab was associated with a higher objective response rate (71% *vs*. 53.5% *vs*. 36.1%) compared to lenvatinib with everolimus and sunitinib respectively. Lenvatinib and pembrolizumab offered significantly higher progression-free survival (23.9 *vs*. 9.2 months, p<0.001) and higher overall survival (HR 0.66, 95% CI 0.49-0.88, p=0.005, OS NR) when compared to sunitinib. This benefit was observed regardless of PD-L1 level or IMDC risk. Lenvatinib and everolimus also offered longer progression-free survival (14.7 *vs*. 0.2 months, p<0.001) compared to sunitinib, but this did not translate into an overall survival benefit. However, toxicity appears to be an issue in this treatment and commonly included hypertension, diarrhoea, elevated lipase and hypertriglyceridaemia. 68.8% of patients in the lenvatinib and pembrolizumab group required dose reduction of lenvatinib and 37.2% of patients discontinued treatment as a result of toxicities. The FDA approved the use of lenvatinib and pembrolizumab in 2021 for treatment of metastatic RCC in 2021. Nonetheless, longer follow up data is required to continue to assess the efficacy and durability of response in this treatment.

DISCUSSION & FUTURE DIRECTIONS

The Selection of First-Line Treatment Regimen in Metastatic ccRCC

The advent of immune checkpoint inhibitors has led to a plethora of new treatment options for metastatic RCC. The approach of combining immune checkpoint inhibitor with a TKI as first-line treatment of metastatic RCC appears promising, yielding higher response rates and improved survival outcomes, demonstrated across multiple phase III trials (**Table 1**). This is supported by the most recent National Comprehensive Cancer Network (NCCN) and European Association of Urology (EAU) guidelines in 2021, which both recommend first-line treatment with immune checkpoint inhibitor in combination with TKI regardless of IMDC risk or alternatively nivolumab and ipilimumab in intermediate and poor IMDC risk (17–23, 27–30). However, most clinical trials compared the efficacy of immune checkpoint inhibitors with sunitinib as the control, which is no longer considered the standard-of-care treatment.

There is no head-to-head trial evidence to compare the efficacy of the various treatment options available including immune checkpoint inhibitors, anti-VEGF therapy or a combination of both. There are multiple factors to consider when selecting first-line treatment for metastatic RCC. The IMDC prognostic risk model remains important in guiding selection of treatment. In favourable-risk disease, first-line treatment options include an anti-angiogenic agent alone or in combination with an immune checkpoint inhibitor, the latter option is favoured as illustrated in both NCCN and EAU guidelines in 2021. In favourable-risk disease, treatment with immune checkpoint inhibitor with TKI offers higher response rate and improved survival outcomes, when compared to treatment with sunitinib alone. In intermediate or poor risk disease, treatment options include ipilimumab and nivolumab or combining an immune checkpoint inhibitor with a TKI. Treatment with immune checkpoint inhibitor and TKI may be favoured in patients who are highly symptomatic with high disease burden and a rapid treatment response is desired, which may be offered by the TKI component of this treatment. Durability of treatment response should also be considered as there is now long-term follow up data to demonstrate the durable response and survival benefits offered by treatment with ipilimumab and nivolumab. In contrast, most clinical trials investigating various treatment regimens with immune checkpoint inhibitor and TKI have shorter follow up and immature long-term data, therefore it is unclear whether this treatment also offers similar durable responses when compared to ipilimumab and nivolumab. Toxicity is also an important consideration given higher rates of immune-related toxicities and requirement for high dose corticosteroid treatment associated with ipilimumab and nivolumab treatment compared to treatment with immune checkpoint inhibitor and TKI.

There are now multiple FDA-approved immune checkpoint inhibitor and anti-VEGF treatment regimens available, which further complicates the decision-making process in selecting treatment for patients with treatment-naïve RCC (**Figure 1**). The regimens used in JAVELIN Renal 101 and IMmotion 151 are unlikely to be preferred options given the lack of overall survival benefit and the latter is not FDA-approved. The treatment regimens used in Keynote-426, Checkmate-9ER and CLEAR all demonstrated impressive response rates but all had various issues with toxicity, and selection should be based on patient characteristics and their other co-morbidities. Lenvatinib and pembrolizumab treatment was associated with higher rates of hypertension and hyperlipidaemia, which may be an issue in patients with cardiovascular co-morbidities. Rates of hepatotoxicity were high in treatment with pembrolizumab with axitinib and nivolumab with cabozantinib, which may be challenging to manage in patients with underlying hepatic impairment. Secondly, histopathological features may guide decision-making as patients with ccRCC with sarcomatoid differentiation were only included in Checkmate-9ER and CLEAR and appear to derive benefit from treatment. Lastly, cost and access to treatment must be considered, which varies internationally. In Australia, only ipilimumab and nivolumab treatment is funded under the Pharmaceutical Benefit Scheme (PBS), none of the treatment regimens with immune checkpoint inhibitor and TKIs are available under PBS access at present.

Current Clinical Trials Investigating Treatment Options in Metastatic ccRCC

The treatment landscape in metastatic RCC continues to evolve with multiple clinical trials investigating the role of combining immune checkpoint inhibitor with targeted agents in both treatment-naïve and treatment-refractory ccRCC (31), summarised in **Table 2**.The current active phase III clinical trials COSMIC-313 and PDIGREE use ipilimumab and nivolumab as the control arm unlike many previous trials which have historically used sunitinib as the control arm. The role of novel targeted agents is investigated in various phase I trials in heavily pre-treated RCC, including ciforadenant, an inhibitor of adenosine A2A receptor, which is expressed on T lymphocytes [NCT02655822].

Tivozanib is a selective and potent TKI that targets the VEGF receptor and demonstrated efficacy in treatment-naive RCC but did not show an overall survival benefit when compared to sorafenib in the phase III trial, TIVO-I (32). Similarly, the phase III trial, TIVO-III demonstrated an improved progression-free survival when tivozanib is used in heavily pre-treated patients with progressive RCC, but this did not translate into an overall survival benefit (33). More recently, the phase I/II trial TiNivo showed that treatment with tivozanib and nivolumab had a higher response rate of 56%, when compared to tivozanib alone (34). The phase III trial, TiNivo-2 is recruiting at present and aims to explore the progression-free survival and overall survival of treatment with tivozanib and nivolumab compared to tivozanib alone in previously treated patients with progressive RCC (35).

Hypoxia-inducible factor-2α (HIF-2α) accumulates abnormally in VHL inactivation, which results in tumour growth and progressive clear-cell RCC (8). Belzutifan is an HIF-2α inhibitor, which demonstrated activity in heavily pre-treated clear-cell RCC in a phase I trial, with an objective response rate of 25% (36). Toxicities included anaemia and hypoxia. The efficacy of belzutifan with cabozantinib is currently investigated in a phase II trial, which is recruiting both treatment-naïve patients and those who progressed with prior immune checkpoint inhibitor treatment [NCT03634540].

Indoleamine 2,3-dioxygenase 1 (IDO1) mediates anergy of effector T cells and contributes to the immunosuppressive tumour microenvironment (37). Therefore, the inhibition of IDO1 is hypothesised to prevent tumour-induced inhibition of T cell activation (37). Epacadostat is an IDO1 inihibitor which demonstrated anti-tumour activity when used with pembrolizumab in a phase I/II trial (38). Unfortunately, this treatment did not demonstrate progression-free or overall survival benefit when used to treat advanced melanoma in a phase III trial (39).

The Search for New Biomarkers in Metastatic RCC

The identification of new predictive biomarkers and treatment targets is important to continue to improve the treatment of metastatic RCC. It has been demonstrated in many pivotal phase III clinical trials that PD-L1 expression is not a predictive biomarker as patients with negative PD-L1 expression also benefit from immune checkpoint inhibitor treatment. This is likely due to variable PD-L1 expression across different metastatic sites (4). However, PD-L1 expression may be a negative prognostic factor and was found to be associated with higher risk of death (40). PD-L1 positivity was also common in those with intermediate or poor risk disease in Checkmate-214 (16).

Neutrophil lymphocyte ratio (NLR) is a measure of inflammation secondary to tumour growth and is the ratio of absolute neutrophil count to absolute lymphocyte count, which has been postulated as a potential biomarker that predicts treatment response to immune checkpoint inhibitor (41, 42). NLR may also be a negative prognostic factor as high NLR is associated with higher risk of death and treatment failure (41, 42). There are small studies

TABLE 2 | Current clinical trials investigating the use of immune checkpoint inhibitor with targeted agents in metastatic RCC.

NCT number	Phase	Histology	Intervention	Control	Primary Endpoint	Treatment Setting	Status
NCT03937219 (COSMIC 313)	III	Clear cell	Nivolumab & ipilimumab + cabozantinib	Nivolumab & ipilimumab only	PFS	First line	Recruiting
NCT03729245	III	Clear cell	Bempegaldesleukin & nivolumab	Sunitinib or cabozantinib	ORR, OS	First line	Recruiting
NCT03873402	III	Clear cell	Nivolumab & ipilimumab	Nivolumab alone	ORR, PFS	First line	Active, not recruiting
NCT04394975	III	Clear cell	Toripalimab & axitinib	Sunitinib	PFS	First line	Recruiting
NCT03260894	III	Clear cell	Pembrolizumab & epacadostat	Sunitinib or pazopanib	ORR	First line	Active, not recruiting
NCT03793166 (PDIGREE)	III	Clear cell	Ipilimumab & nivolumab followed by maintenance nivolumab & cabozantinib	Ipilimumab & nivolumab followed by maintenance nivolumab only	OS	First line	Recruiting
NCT03289962	I	Multiple cancers including ccRCC	Autogene cevumeran & atezolizumab	NA	DLT RP2D Adverse events	Subsequent line	Recruiting
NCT02964013	I	Multiple cancers including ccRCC	Vibostolimab (Anti-TIGIT antibody) & pembrolizumab	NA	DLT Adverse events	Subsequent line	Recruiting
NCT02655822	I	ccRCC	Ciforadenant (A2AR inhibitor) & atezolizumab	NA	DLT ORR Adverse events	Subsequent line	Recruiting
NCT02754141	I	Multiple cancers including ccRCC	BMS-986179 (CD73 inhibitor) & nivolumab	NA	Adverse events	Subsequent line	Recruiting

OS, overall survival; PFS, Progression-free survival; ORR, Objective response rate; DLT, Dose-Limiting Toxicites; RP2D, Recommended Phase 2 Dose.

to suggest that reduction of NLR pre-treatment and after treatment is associated with improved outcomes in those treated with immune checkpoint inhibitor in advanced RCC (41, 42). However, the value of NLR as a biomarker requires further investigation.

PBRM1 is a possible biomarker, which is a gene that plays a role in remodelling of chromatin (43, 44). PBRM1 mutations occur less frequently in tumours in RCC with high levels of tumour-infiltrating lymphocytes (TILs), which is associated with greater response to nivolumab in an analysis of Checkmate-025 (45). Further, T-cell immunoglobulin-3 (TIM-3) may be found expressed on TILs and contributes to suppression of T-cell mediated immune responses against tumour proliferation hence reduced response to immune checkpoint inhibitors (45, 46). Further investigation into the role of PBRM1 and TIM-3 in predicting treatment response is required. There are currently multiple early phase trials investigating the role of anti-TIM-3 agents in combination with immune checkpoint inhibitors in various cancers including RCC [NCT02817633, NCT03708328].

Immune Checkpoint Inhibitor Treatment of Metastatic nccRCC

nccRCC is a diverse group of RCCs with various histological subtypes that are vastly different but treated as one group due to rarity of the individual subtypes (2). The most common subtypes of nccRCC include papillary (5 to 10%), chromophobe (5%) and unclassified (< 5%), rarer subtypes including renal medullary, MiT family translocation and SDH-deficient nccRCC all constitute less than 1% of nccRCC (2). There is a lack of evidence to guide treatment of metastatic nccRCC as most clinical trials only included patients with ccRCC. Retrospective studies investigating outcomes in nccRCCs tend to be dominated by patients with papillary and chromophobe subtypes and few with collecting duct, medullary and translocation-associated nccRCC are included as these subtypes are even rarer (2, 46, 47). Overall, nccRCCs demonstrate less response to targeted therapy with anti-VEGF and mTOR inhibitors and has poorer prognosis in survival outcomes when compared to ccRCCs (46, 47). The most recent EAU and NCCN guidelines recommend enrolment of patients with nccRCC onto clinical trials if possible. Targeted therapy such as anti-VEGF TKIs are recommended as first-line treatment of papillary, chromophobe, translocation and unclassified nccRCC whereas platinum-based chemotherapy is recommended for treatment of medullary and collecting duct RCC (29, 30).

nccRCC often have positive PD-L1 expression, which is associated with more advanced disease and poorer prognosis, similar to in ccRCC (48, 49). There is some evidence demonstrating response of nccRCC to immune checkpoint inhibitors but data is limited and mostly retrospective in nature (49, 50). The Keynote-427 phase II trial recruited patients with nccRCC into cohort B of the study, of which 71.5%, 12.7% and 15.8% had papillary, chromophobe and unclassified subtypes respectively (51). This trial demonstrated activity of pembrolizumab in nccRCC with an objective response rate of 24.8% and 81.5% of responders had a durable response of greater than 6 months. Those with papillary and unclassified histology had the highest response rates compared to chromophobe histology (51). It is hypothesised that chromophobe nccRCC are less immunogenic with less immune cell infiltration and therefore have a lower response rate to immune checkpoint inhibitor treatment in comparison to other nccRCC subtypes (52). Checkmate-920 is the first prospective phase III trial to demonstrate the efficacy of nivolumab and ipilimumab in first-line treatment of advanced nccRCC. This trial recruited patients with various nccRCC histological subtypes, including papillary (34.6%), chromophobe (13.5%), unclassified (42.3%) and rarer subtypes including collecting duct (3.8%), medullary (1.9%) and translocation (3.8%) (53). Preliminary results showed an overall objective response rate of 19.6%, progression-free survival of 3.7 months and overall survival of 21.2 months. There were no new safety signals identified with regard to immune-related toxicities when compared to the ccRCC group. SUNIFORECAST is a phase II trial that investigates the efficacy of ipilimumab and nivolumab compared with sunitinb in the first-line treatment of nccRCC and is currently recruiting [NCT03075423]. Data from further prospective studies are required to directly compare the clinical benefit of immune checkpoint inhibitor in the treatment of ccRCC and various subtypes of nccRCC.

CONCLUSION

The treatment landscape of metastatic RCC has evolved in the last decade with the rise of immune checkpoint inhibitors in addition to the development of novel TKIs. This has resulted in the improvement of prognosis and survival for patients with metastatic RCC. However, given the rising incidence of metastatic RCC and the lack of evidence to guide treatment of nccRCC, there is a strong need for ongoing research in identification of new biomarkers and development of novel targeted agents to overcome persistent challenges posed by tumour resistance and to guide treatment decisions.

AUTHOR CONTRIBUTIONS

IT contributed to the writing and completion of this manuscript. AS contributed to the conception of the research topic and the supervision of this project. All authors contributed to the article and approved the submitted version.

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A Retrospective Study of First-Line Therapy Involving Immune Checkpoint Inhibitors in Patients With Poor Risk Metastatic Renal Cell Carcinoma

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Purpose: Patients with International Metastatic RCC Database Consortium (IMDC) poor risk metastatic renal cell carcinoma (mRCC) rarely respond to first-line tyrosine kinase inhibitors (TKIs) including sunitinib, and carries a very poor prognosis. In recent years, combination therapy involving immune checkpoint inhibitors (ICIs) have demonstrated superior efficacy to sunitinib in poor risk disease.

Materials and Methods: In a retrospective study using a cancer chemotherapy registry, 206 consecutive patients with mRCC in the first-line setting were identified between Oct 2019 and Dec 2020. Sixty-one patients had a poor risk mRCC, and were treated with TKI monotherapy (n=36), nivolumab plus ipilimumab (n=16), or pembrolizumab plus axitinib (n=9). Endpoints included overall survival (OS), progression-free survival (PFS), response rate (RR), and safety.

Results: Patients' median age was 61 years and the median number of risk factors was 3 (range, 3-5). During a median 23.0 months of follow-up, the median OS was 24.3 months with ICI-based combinations and 14.8 months with TKI monotherapy, and the median PFS periods were 9.3 months and 3.4 months, respectively. An objective response occurred in 60% of the patients receiving ICI-based combinations and in 19% of those receiving TKI monotherapy (P=0.001). In the multivariate regression model, number of IMDC risk factors and the ICI-based combination therapy were independent prognostic factors for PFS. All-causality grade 3 or 4 adverse events were 44% for ICI-based combinations and 50% for TKI monotherapy.

Conclusions: Among patients with poor risk mRCC, first-line ICI-based therapy showed significantly longer OS and PFS, as well as a higher RR, than TKI monotherapy.

Keywords: immunotherapy, immune checkpoint inhibitors, renal cell carcinoma, IMDC poor-risk, overall survival, sunitinib

INTRODUCTION

Over the past two decades, tyrosine kinase inhibitors (TKIs) targeting the vascular endothelial growth factor receptor (VEGFR), including sunitinib (1) and pazopanib (2), are standards-of-care for patients with clear cell metastatic renal cell carcinoma (mRCC). However, some patients who receive first-line TKIs do not achieve clinical response, and show a rapid progression (3). Patients with an intrinsic resistance to TKIs, or poor risk disease, are supposed to have a limited benefit from first-line sunitinib, and although temsirolimus was suggested as an option (4), those with poor risk mRCC had a grim prognosis (3). These patient subgroups probably differ both clinically and biologically, and the International Metastatic RCC Database Consortium (IMDC) derived a risk model in the era of TKIs from a large patient cohort (5), with 6 independent predictive factors of poor survival including a performance status, an interval from time of RCC diagnosis to systemic therapy, hemoglobin level, calcium, neutrophil and platelet counts.

First-line treatment for mRCC has expanded in recent years to include immune checkpoint inhibitors (ICIs) including nivolumab plus ipilimumab (6) and pembrolizumab plus axitinib (7). As a result, current guidelines recommend these ICI-based doublets in patients with mRCC considered intermediate or poor risk groups, whereas for all IDMC risk categories pembrolizumab plus axitinib has emerged as a preferred standard regimen (8). In Korea, nivolumab plus ipilimumab and pembrolizumab plus axitinib were approved for the first-line therapy in mRCC in 2018 and 2019, respectively. Since the ICI-based doublets were not fully reimbursed by the national health insurance system before Sep 2021, our patients received the regimens at the discretion of the treating medical oncologists based on clinical and/or economic judgment. In patients not eligible for ICIs, or who cannot afford to the drug cost, VEGFR TKIs including sunitinib or pazopanib were still offered to those with poor risk disease.

Considering the grim prognosis of poor risk mRCC patients, and in an effort to generate real-world data in Korean mRCC patients, we performed a retrospective study using a prospectively collected cancer chemotherapy registry. Because prospectively-designed, randomized controlled trials (RCTs) comparing these ICI-based doublets are lacking, retrospective, or real-world studies seem to be an important source of data to allow the choice of an optimal treatment, enhance patient counseling, and generate hypothesis for future studies.

METHODS

In the present single-center, retrospective study, we collected and reviewed follow-up patient data from our cancer registry. Written informed consent was given by all patients prior to receiving first-line systemic therapy for their mRCC, according to institutional guidelines. The study protocol was reviewed and approved by the Samsung Medical Center (SMC, Seoul, Korea) institutional review board (SMC IRB no. 2021-08-054). The criteria for case inclusion were as follows: (1) histologically confirmed diagnosis of clear cell carcinoma arising from kidney, (2) presence of metastatic disease, (3) no prior systemic therapy except for adjuvant treatments, (4) poor risk disease, and (5) availability of clinical data at the time of beginning therapy and follow-up. We excluded patients who were enrolled in clinical trials to ensure the choice of therapy was at the discretion of the treating doctors. All the data was prospectively recorded and only the survival data was updated at the time of analyses.

IDMC poor risk was defined according to the IMDC criteria (5): (1) Karnofsky performance status <80%, (2) less than 1 year from time of RCC diagnosis to systemic therapy, (3) anemia (hemoglobin level <lower limit of normal [LLN], 12 g/dL), (4) hypercalcemia (corrected calcium >upper limit of normal [ULN], 10.2 mg/dL), (5) neutrophilia (neutrophil count >ULN, $7.0x10^9$/L), and (6) thrombocytosis (platelet count >ULN, $400x10^9$/L). According to the number of risk factors, patients were categorized into favorable (0), intermediate (1 or 2 factors), and poor (3 or more factors) risk groups. All patients received first-line therapy involving TKI monotherapy (sunitinib or pazopanib), nivolumab plus ipilimumab, or pembrolizumab plus axitinib. Dosages and therapy schedules of each regimen were determined according to the approved guidelines. Therapy was continued until disease progression or lack of clinical benefit, withdrawal of consent, justifiable withdrawal at the investigator's discretion, or toxicity. Toxicities were graded according the National Cancer Institute (NCI) criteria (CTCAE). The dosage of the subsequent cycles was adjusted according to the toxic effects that developed during the preceding cycle. After the first-line therapy had failed, second-line therapy was recommended to all the patients if their performance status was preserved. According to the guidelines and department policies, all tumor measurements were assessed after every 3 months of therapy, by using an abdominopelvic computed tomography (CT) scan and other tests that were used initially to stage the tumor. Tumor response was evaluated according to the Response Evaluation Criteria for Solid Tumors (RECIST).

The primary endpoint of the present study was overall survival (OS). Secondary endpoints included progression-free survival (PFS), response rates (RR), and safety. The starting of OS and PFS was the first day of therapy. PFS and OS were estimated according to the Kaplan-Meier method and the statistical significance of survival curves between groups was tested with a log-rank test. To examine the impact of clinical and treatment variables on the outcomes of therapy, multivariate Cox regression models were used with covariates including age (below vs. ≥ median), gender, previous nephrectomy, presence of other histologic subtypes than clear cell carcinoma, lactate dehydrogenase (LD), weight loss (>5%) before therapy, number of involved sites (one vs. ≥2), sites of metastases (liver, bone), baseline number of IMDC risk factors (3 vs. >3), and therapy regimens. The potential presence of interaction effects between baseline parameters was tested by defining product terms for the respective factors in a regression model. All P values were two-

sided, with $P < 0.05$ indicating statistical significance. Analyses were performed using the R for Windows v2.11.1 software (R Core Team, Vienna, Austria; http://www.r-project.org).

RESULTS

We identified a total of 206 patients who were consecutively treated with first-line therapy for mRCC at the medical oncology department of SMC between Oct 2019 and Dec 2020. Among them, 61 patients were identified to have a poor risk mRCC (**Figure 1**). Fifty-nine percent (n=36) of patients received TKI monotherapy, and others (n=25) received ICI-based combinations (nivolumab plus ipilimumab, n=16; pembrolizumab plus axitinib, n=9). Baseline patient characteristics are listed in **Table 1**. The median number of IDMC risk factors was 3 (range, 3-5), and most commonly observed risk factors included anemia (84%) and the interval between diagnosis and therapy (71%). Forty-three (71%) patients had prior nephrectomy. Most common sites of metastases included lung and lymph nodes. At the time of analysis (Dec 2021), 57 (93%) patients had discontinued their first-line therapies.

Patients received for a median of 3.8 months (95% CI, 3.1-4.5) of first-line therapy (**Table 2**). The most common reason for therapy discontinuation was progressive disease (75%). Overall, both TKIs and ICI-based combinations were generally well tolerated. Among 36 patients treated with TKI monotherapy, one patient discontinued therapy due to the development of acute myocardial infarction. In 25 ICI-treated patients, 4 patients discontinued therapy due to toxicities: grade 3 polyneuropathy (n=1), grade 4 hepatitis (n=1), grade 4 pneumonitis (n=1), and sudden death (n=1). A 58-year-old male patient was found dead at home in the midst of 7th cycle of pembrolizumab plus axitinib, with no clinical evidence of progression or adverse events demonstrated.

Among 61 patients with poor risk mRCC, 2 patients could not be evaluated for clinical responses because of early discontinuation of therapy. Objective responses to first-line therapy were noted in 29 patients (RR, 48%; 95% CI, 35-60%), including 4 complete responses seen in patients with ICI-based combination therapies. Patients who received TKI monotherapy were significantly less likely to respond to therapy (19% vs. 60%; P=0.001) compared to those who were treated with ICI-based combinations. RR was not significantly influenced by age, gender, weight loss, IMDC risk, or metastatic sites.

With a follow-up duration of 23.0 months (95% CI, 22.1-24.4), the estimated median PFS and OS were 5.7 months (95% CI, 2.8-8.5) and 19.7 months (95% CI, 13.0-26.4), respectively. Both PFS (9.3 vs. 3.4 months; **Figure 2A**) and OS (24.3 vs. 14.8 months; **Figure 2B**) were longer in patients receiving ICIs than those receiving TKI monotherapy. In the univariate model, the estimated PFS was significantly longer for patients who received ICI-based combinations (P=0.001), and who had 3 risk factors (P=0.022). OS also was longer for patients who had 3 risk factors (P=0.042). However, no statistically significant difference in the OS was observed between ICI combinations and TKI monotherapy (P=0.162). A subsequent multivariate regression model revealed that independent prognostic factors for PFS were number of IMDC risk factors and the ICI-based combination therapy (**Table 3**). The presence of >3 IMDC risk factors was the only poor prognostic factor for OS.

For exploratory purposes, we compared PFS and OS in 25 patients treated with ICI-based combinations according to regimens given. No statistically significant differences in the median PFS (9.0 and 9.4 months, respectively) and OS (21.9 and 25.1 months, respectively) were observed between patients who received nivolumab plus ipilimumab and pembrolizumab plus axitinib. After first-line failure, second-line therapy was given to more than half of patients (n=34). Specifically, most patients received second-line TKIs (sunitinib, n=12; cabozantinib, n=8; axitinib, n=4), and novel therapeutics were given in 10 patients in the context of clinical trials. OS was longer in patients able to receive second-line therapy (25.3 vs. 12.9 months) than those without further therapy.

DISCUSSION

The main purpose of the present retrospective study was to investigate the real-world outcomes of patients with poor risk mRCC treated with different first-line therapy regimens. After

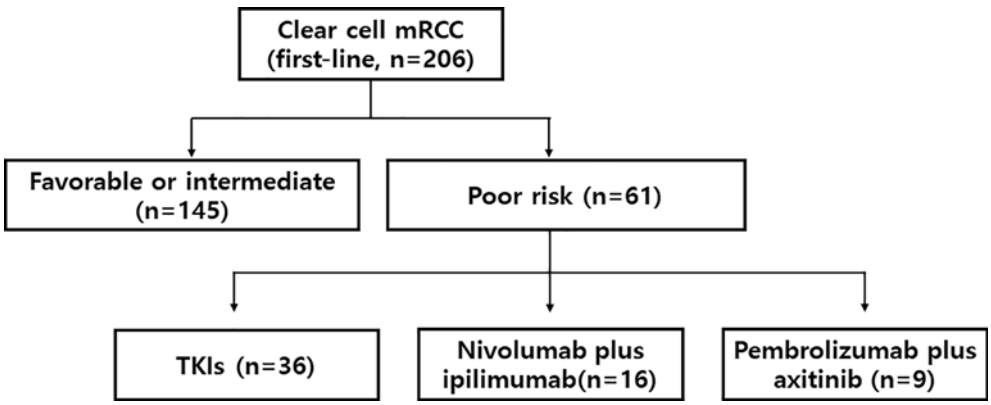

FIGURE 1 | Study flow. mRCC denotes metastatic renal cell carcinoma. TKI denotes tyrosine kinase inhibitor.

TABLE 1 | Patient characteristics of patients with poor risk, metastatic, clear cell renal cell carcinoma.

	All patients (n=61)	Tyrosine kinase inhibitors (n=36)	Checkpoint inhibitors (n=25)
Age, years			
Median (range)	63 (37-84)	66 (43-84)	58 (37-79)
Gender			
Male	47 (77%)	28 (78%)	19 (76%)
Female	14 (23%)	8 (22%)	6 (24%)
Prior nephrectomy	43 (71%)	30 (83%)	13 (52%)
Mixed histology	15 (25%)	8 (22%)	7 (28%)
Lactate dehydrogenase, IU/L			
Median (range)	284 (118-1,618)	284 (125-643)	283 (118-1,618)
Weight loss (>5%)	27 (44%)	17 (47%)	10 (40%)
No. of IMDC risk factors			
3	47 (77%)	28 (78%)	19 (76%)
4 or more	14 (23%)	8 (22%)	6 (24%)
IMDC risk factors			
Interval diagnosis/therapy <1y	43 (71%)	25 (69%)	18 (72%)
Karnofsky PS <80%	26 (43%)	14 (39%)	12 (48%)
Anemia	51 (84%)	30 (83%)	21 (84%)
Hypercalcemia	20 (33%)	11 (31%)	9 (36%)
Neutrophilia	18 (30%)	9 (25%)	9 (36%)
Thrombocytosis	32 (53%)	22 (61%)	10 (40%)
No. of metastatic sites			
1	27 (44%)	17 (47%)	10 (40%)
2 or more	34 (56%)	19 (53%)	15 (60%)
Metastatic sites			
Lymph nodes	22 (36%)	11 (31%)	11 (44%)
Lung	47 (77%)	26 (72%)	21 (84%)
Liver	8 (13%)	3 (8%)	5 (20%)
Bone	15 (25%)	9 (25%)	6 (24%)
Pancreas	9 (15%)	8 (22%)	1 (4%)
Brain	5 (8%)	2 (6%)	3 (12%)
Therapy regimen			
Sunitinib	29	29	
Pazopanib	7	7	
Nivolumab/ipilimumab	16		16
Pembrolizumab/axitinib	9		9

IMDC denotes the International Metastatic RCC Database Consortium. PS denotes performance status.

regulatory approvals of first-line ICI-based therapy, significant prolongations of both PFS (9.3 vs. 3.4 months) and OS (24.3 vs. 14.8 months) were observed when compared to TKI monotherapy. This is consistent with the findings of the published trials of ICI-based first-line therapy (6, 7). Although interpretation of the present findings are limited by its retrospective nature and small sample size, the results provide a piece of evidence that patients with a poor-risk mRCC may derive an indisputable benefit from ICI-based combinations. Although it would be difficult to choose best first-line regimen from the present study or others, nivolumab plus ipilimumab and pembrolizumab plus axitinib provided similar outcomes.

Despite recent advances in the treatment of patients with clear cell mRCC, the prognosis of the IDMC poor risk patients remains challenging. Although current guidelines recommend first-line treatment with ICI in combination with TKI or nivolumab plus ipilimumab in this patient population (8), there remains controversy surrounding the choice of therapy regimens for poor risk disease. There is no head-to-head trial comparing the efficacy of the therapy options available including ICIs, TKIs, or a combination

TABLE 2 | Therapy compliance and safety.

	All patients (n=61)	Tyrosine kinase inhibitors (n=36)	Checkpoint inhibitors (n=25)
Therapy duration, mo			
Median	3.8	3.1	7.4
95% confidence interval	3.1-4.5	2.7-3.5	3.9-11.0
Reasons for discontinuation			
Progressive disease	46 (75%)	31 (86%)	15 (60%)
Toxicity	5 (8%)	1 (3%)	4 (16%)
Withdrawal	1 (2%)	1 (3%)	0
Physician recommendation	5 (8%)	2 (6%)	3 (12%)
Ongoing	4 (7%)	1 (3%)	3 (12%)
Overall grade 3 or 4 toxicity	29 (48%)	18 (50%)	11 (44%)
Corticosteroids use	7 (12%)	2 (6%)	5 (20%)
Treatment-related deaths	1 (2%)	0	1 (4%)

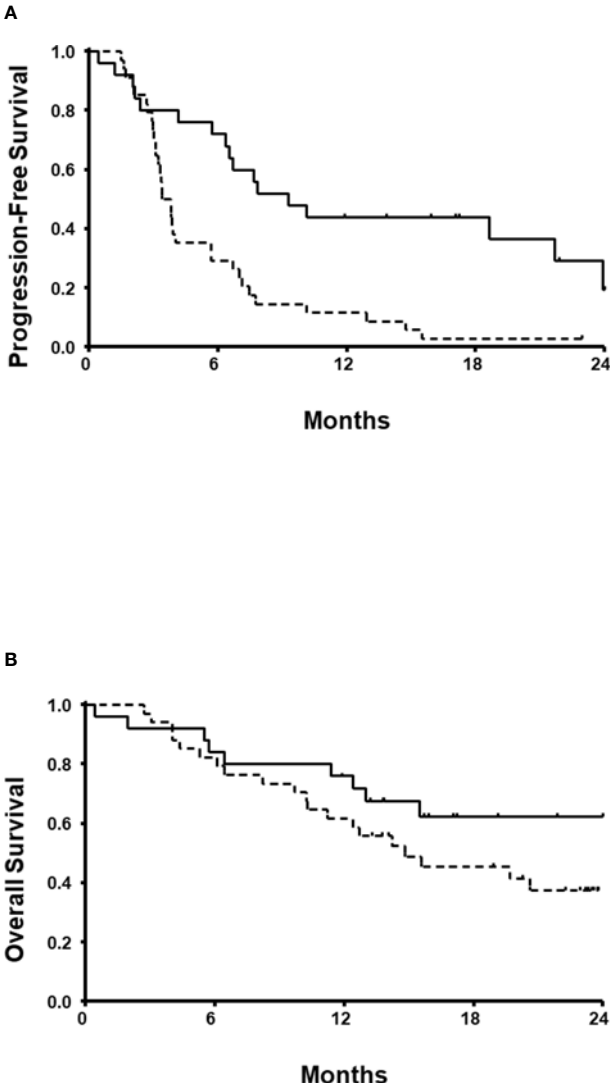

FIGURE 2 | Progression-free survival **(A)** and overall survival **(B)**. Solid lines denote patients who received immune checkpoint inhibitors. Dotted lines denote patients who received tyrosine kinase inhibitor monotherapy.

of both. Furthermore, in the IMDC retrospective study, there were no significant differences in first-line outcomes between nivolumab plus ipilimumab and ICI plus VEGFR TKIs (9). ICI plus TKI may be preferred in patients with highly symptomatic disease and a rapid clinical response is required, which may be offered by the TKI component of the regimen. One may consider a durable treatment response to be important as there is long-term follow-up data to demonstrate the durable response and survival benefit with nivolumab plus ipilimumab (6). Toxicity is also an important consideration given the balance between higher rates of immune-

TABLE 3 | Multivariate analyses according to baseline clinical factors and therapy.

	Progression-free survival	Overall survival
No. of risk factors=3 vs. >3	HR 0.447	HR 0.441
	95% CI 0.220-0.906	95% CI 0.196-0.992
	P=0.026	P=0.048
Checkpoint inhibitors vs. TKIs	HR 0.339	HR 0.567
	95% CI 0.182-0.630	95% CI 0.258-1.246
	P=0.001	P=0.158

TKI denotes tyrosine kinase inhibitors.

related adverse events associated with ICIs and the possibility of symptomatic deteriorations with TKIs.

In addition to clinical factors, appropriate patient selection based on molecular markers is one of the most extensively studied areas in clinical research. While PD-L1 expression is not considered a predictive marker as patients with PD-L1 negative tumors also benefit from ICI therapy, and the heterogeneity in PD-L1 testing methods adds complexity to this issue. Extensive work is ongoing to identify possible molecular markers, including the tumor mutation burden, immune infiltrates in the tumor microenvironment, or gene signatures, that could be related to sensitivity or resistance to ICIs (10), as well as specific genomic subtypes harbored in different risk groups (11).

More recently, more than a few novel combination therapy regimens have demonstrated improved survival outcomes (12–15), all of which compared the efficacy of ICI-based therapy with sunitinib as the control, which is no longer considered the standard of care in this patient population. As seen in these clinical trials involving therapeutic strategies, further advances in the treatment of poor risk mRCC will only be achieved with better patient selection. Emerging science and the knowledge of disease may further guide us to enhance individualized therapy for patients with mRCC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.tf

AUTHOR CONTRIBUTIONS

All authors contributed conception and design of the study. GK, KK, SYP, CK, BP, JC, WS, MK, HS, HGJ, BJ, SS, SJ, HL, and SHP acquired the clinical data. HJ and SHP conducted the statistical analysis. HJ, JH, HK, HRK, and SHP analyzed and interpreted the data. HJ and SHP drafted the manuscript. All authors read and approved the submitted version.

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CASE REPORT
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Case Report: New Onset Lymphadenopathy After Immune Checkpoint Inhibitor Therapy Presents a Clinicopathological and Radiological Challenge

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The use of immune-checkpoint inhibitor (ICI) therapy has significantly improved patient outcomes in a wide variety of cancers and has become a cornerstone in the treatment of renal cell carcinoma. However, ICI treatment has the potential to cause a variety of immune-related adverse events (irAEs) that can affect any tissue or organ. This report describes the diagnostic dilemma of a patient with both RCC and diffuse large B-cell lymphoma who developed acute onset of fever and diffuse lymphadenopathy following treatment with combined ipilimumab and nivolumab. While diagnostic considerations included worsening lymphoma, hyperprogression of RCC, sarcoid-like reaction from immunotherapy, and fungal infection, his lymphadenopathy eventually resolved with treatment for histoplasmosis and discontinuation of immunotherapy. Despite only receiving two doses of immunotherapy, he has not required additional systemic therapy for RCC. This case demonstrates both the effectiveness of ICI therapy and the need for multidisciplinary approach to potential irAEs.

Keywords: immune checkpoint inhibitors, lymphadenopathy, renal cell carcinoma, diffuse large B-cell lymphoma, sarcoid-like reaction, histoplasmosis, immune-related adverse event

INTRODUCTION

Immune-checkpoint inhibitor (ICI) therapy has become integral in the treatment of renal cell carcinoma (RCC) and many other malignancies. Despite the striking survival benefit of these agents, immune-related adverse events (irAEs) can occur in a subset of patients with a wide range of presentations and severity that can be challenging to diagnose and treat. Several management algorithms and guidelines have been developed to guide treatment of irAEs (1, 2). Currently, there are no validated biomarkers to guide treatment selection or stratify patients at risk for developing irAEs. Due to the importance of immediate intervention, providers need to recognize the myriad of ways irAEs can manifest and the diagnostic challenges they pose.

In this report, we present a patient with a history of both RCC and diffuse large B-cell lymphoma (DLCBL) who presents with fever and diffuse FDG-avid lymphadenopathy after treatment with

immune checkpoint inhibitors. This case represents a diagnostic challenge due to the broad differential diagnosis including malignant, infectious, and immunotherapy-related causes, and it highlights the importance of a multidisciplinary approach to lymphadenopathy following ICI treatment. The history of both RCC and DLBCL supports a previously identified link between RCC and hematologic malignancies as well as the need to further evaluate the genetic and environmental factors associated with their co-occurrence.

CASE PRESENTATION

A 65-year-old man was initially diagnosed with clear cell renal cell carcinoma (ccRCC) in 2002 after presenting with gross hematuria and underwent left radical nephrectomy showing a 5.5-cm nucleolar grade 2 ccRCC with renal vein invasion, staged as pT3B according to the AJCC sixth edition staging criteria. He was later diagnosed in 2019 with stage II DLBCL, germinal center type with right inguinal and right femoral involvement. During his lymphoma evaluation, he was found to have an incidental 3.2-cm mass in the medial aspect of the right mid kidney and a 1.1-cm mass in the interpolar right kidney suspicious for RCC. Core needle biopsy confirmed recurrent ccRCC.

He received three cycles of R-CHOP chemotherapy and consolidative radiotherapy (RT) for his DLBCL with a posttreatment positron emission tomography/computed tomography (PET/CT) showing complete resolution of previously noted lymphadenopathy. He underwent right partial nephrectomy of the right mid kidney mass and cryoablation of the interpolar right kidney mass. Subsequent imaging showed concern for metastatic disease with a PET/CT scan in June 2020 revealing bilateral pulmonary nodules as well as lesions in the right hepatic lobe, adjacent to the gallbladder, and an L3 lytic lesion. Ultrasound-guided liver biopsy confirmed metastatic ccRCC.

He was treated with stereotactic body radiation therapy (SBRT) to L3 with 2,400 cGy given he was symptomatic with pain at the site of his L3 metastasis. He was subsequently referred to Medical Oncology at the Sidney Kimmel Cancer Center at Johns Hopkins. His disease risk status was intermediate per the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) risk groups. In this context, he initiated treatment for metastatic ccRCC with doublet immune-checkpoint inhibitors (ICIs) using the combination of ipilimumab 1 mg/kg and nivolumab 3 mg/kg, which is an FDA-approved regimen based on the phase 3 Checkmate-214 clinical trial (3).

Following his second dose, he presented to the emergency department with non-neutropenic fevers and rigors without a clear source. He continued to have intermittent fevers despite regular acetaminophen given to treat a potential reaction to ipilimumab plus nivolumab. He was admitted to the hospital after returning to the emergency department with persistent fevers, acute kidney injury, and transaminitis. He remained febrile despite broad-spectrum antibiotics and isavuconazole given in the context of his pulmonary nodules. CT imaging showed new generalized progressive lymphadenopathy in the chest, abdomen, and pelvis. PET/CT scan showed extensive, intensely FDG avid lymphadenopathy as well as new diffusely avid splenomegaly (**Figure 1**). In view of this, the differential diagnosis included recurrence of DLBCL, hyperprogression of metastatic ccRCC, immune-related toxicity with sarcoid-like reaction, or other causes of inflammatory and infectious lymphadenopathy. Left axillary fine needle aspirate (FNA) and core biopsy showed granulomatous inflammation with negative stains for mycobacteria (Ziehl–Neelsen stain) or fungal organisms (GMS stain) and were also negative for involvement with RCC, lymphoma, or other malignancy (**Figure 2**). An extensive infectious disease evaluation was negative including T-spot, acid-fast bacillus (AFB) blood culture, coccidioides serologies, Q fever serologies, Bartonella serologies, blood histoplasma antigen, and histoplasma antibody. Given the possibility of histoplasmosis

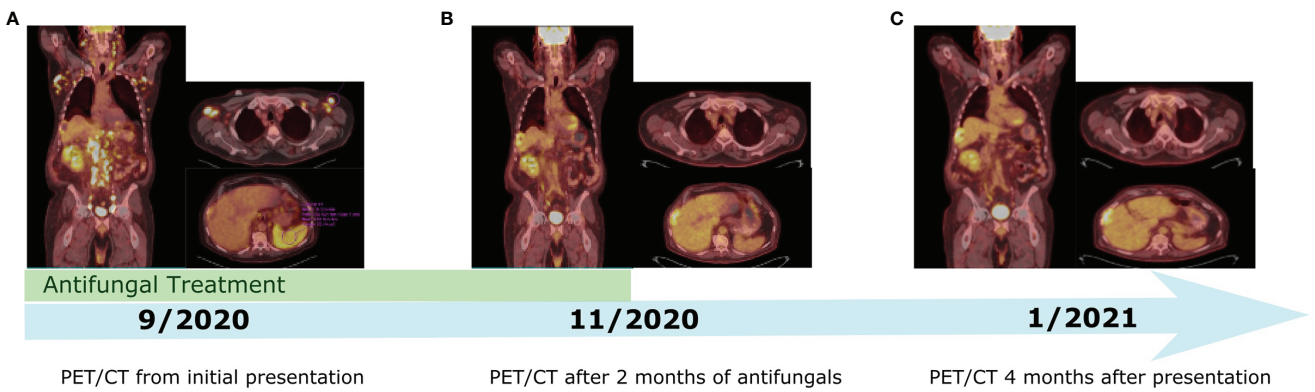

FIGURE 1 | Timeline of PET/CT imaging throughout the patient's course. **(A)** PET/CT from Initial presentation with fever and diffuse FDG avid lymphadenopathy. **(B)** PET/CT showing significant decrease in size and metabolic activity of previously enlarged lymph nodes after two months of antifungal treatment. **(C)** PET/CT showing complete resolution of lymphadenopathy 4 months after initial presentation.

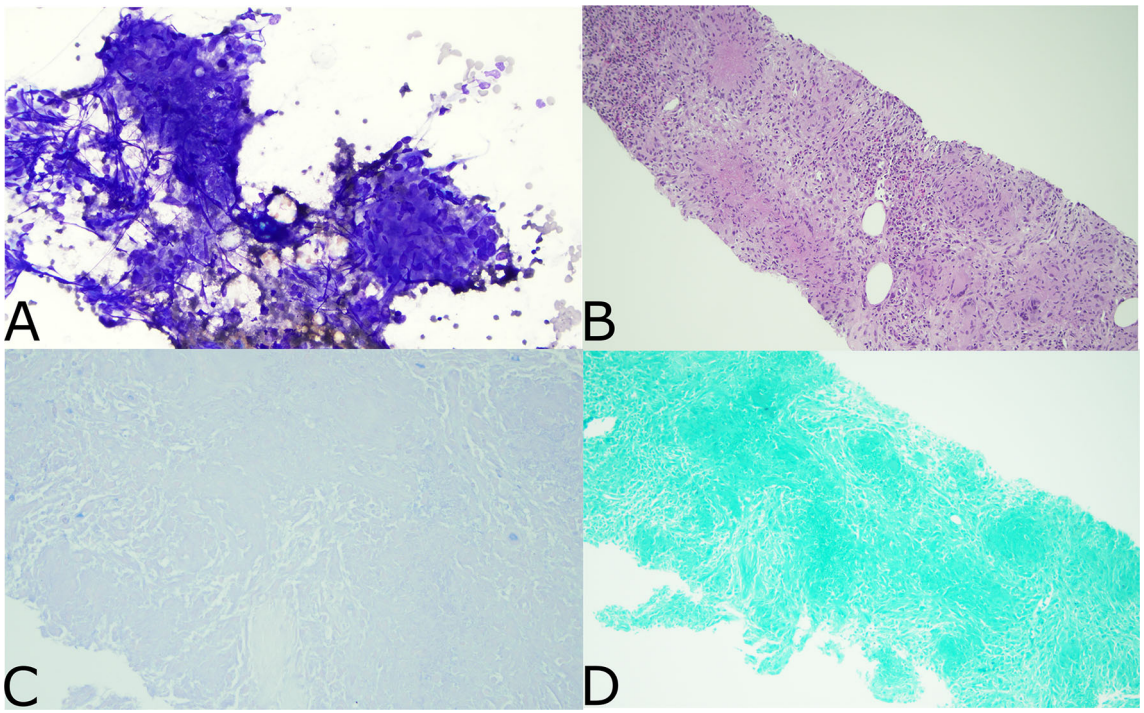

FIGURE 2 | Pathology from Left axillary FNA. **(A)** Aspirated material of a lymph node shows multiple granulomas. They are charecterized by syncytium cytoplasm and spindled, elongated, carrot shaped and barefoot shaped nuclei (Diff-Quik stain, x200). **(B)** This small core biopsy shows numerous necrotizing granulomatous inflammation effacing the entire lymphoid tissue. The granulomas are characterized by round to oval structures consisting of epithelioid histiocyties with focal central necrosis. Scattered lymphocytes and multinucleated giant cell are noted (H&E stain X100). **(C)** Ziehl neelsen stain is negative for mycobacterial organisms (Ziel neelsen stain X400). **(D)** GMS stain is negative for fungal microorganisms (GMS stain X200).

as the cause of his granulomatous inflammation in the context of farm exposures and splenic granulomata, he was transitioned from isavuconazole to posaconazole for a prolonged course despite negative blood histoplasma antigen and antibodies. While a sarcoid-like reaction secondary to ICIs was also considered, steroids were not initiated given normal pulmonary function tests (PFTs) and lack of parenchymal manifestations. His fevers resolved after 1 week of antifungal therapy. He was continued on posaconazole for 3 weeks and transitioned to itraconazole due to lower-extremity edema. Itraconazole was continued until a follow-up PET/CT 2 months after presentation showed a marked decrease in the size and metabolic activity of his previously enlarged cervical, thoracic, abdominal, and pelvic lymph nodes (**Figure 1**). Repeat PET/CT 4 months after presentation showed complete resolution of his lymphadenopathy, which was ultimately attributed to disseminated histoplasmosis (**Figure 1**). Treatment with ICI was discontinued given the small possibility that his presentation could be immune-related. Given he had stable disease and there was concern for ongoing infection, he was monitored off therapy. His RCC remained stable without treatment besides a growing right chest wall metastatic lesion for which he received additional radiotherapy in February 2021. He has remained off systemic therapy without fevers, and his most recent surveillance CT imaging showed stable disease in August 2021 consistent with a treatment-free survival of approximately 12 months since his last dose of ICI. Considering his personal history of two malignancies, he underwent germline genetic testing which was negative for pathogenic alterations.

DISCUSSION

As in this patient with both RCC and DLBCL, multiple studies have shown an association between RCC and hematologic malignancies (4–10). Concurrent RCC and hematologic malignancies occur mostly in men, typically involving lymphoid malignancies, and the hematologic malignancy usually occurs concurrently or prior to RCC (8, 10). Prior epidemiologic studies have shown a higher than expected occurrence of RCC and non-Hodgkin lymphoma (NHL) with observed-to-expected rates of RCC in the NHL population of 1.86 and observed-to-expected rates of NHL in the RCC population of 2.67 (9). Another SEER analysis observed a significantly higher occurrence of NHL and RCC than expected (4). In this study, the number of RCC cases after an NHL diagnosis was significantly higher than expected with an observed-to-expected ratio of 1.51 (95% CI 1.36–1.66), but the

observed number of cases of NHL after RCC was not significantly different.

The mechanism of this association is speculative, but it may be due to common genetic mutations, environmental factors, or immune dysregulation (5–7, 10, 11). The study of relatives of patients with concurrent RCC and hematologic malignancy has suggested a hereditary association given greater lymphoid-predominant malignancy in men within these families and a suggestion of age-of-onset anticipation (12). However, specific mutations linking these concurrent malignancies have not been found thus far. While hereditary renal cancer syndromes such as those involving the Von Hippel–Lindau tumor-suppressor gene have been identified, these syndromes do not typically lead to an increased risk of hematologic malignancies (13). However, there are abnormalities of 3p chromosome that have been reported in B-cell lymphomas (14). This is relevant considering that the Von Hippel–Lindau (VHL) gene is located on the 3p chromosome, and loss of 3p is considered a landmark driver in disease evolution (15, 16). Furthermore, patients with Cowden syndrome characterized by the presence of PTEN germline alterations are at risk for the development of RCC and lymphomas, but this pathway has not been shown to be a definitive common pathway between the two malignancies (11, 17). Others have suggested that dysregulation in immune surveillance in the context of lymphoma may predispose individuals to RCC (4). Further studies are needed to more thoroughly examine the association between RCC and lymphoid malignancies.

Our patient presented with diffuse lymphadenopathy after 2 cycles of ICI combination therapy. In recent years, several small studies have reported the association between ICI and a sarcoid-like reaction often presenting as bilateral mediastinal or hilar lymphadenopathy (18–23). While it has rarely been reported with PD-1/PD-L1 inhibitors, ipilimumab (a monoclonal antibody targeting CTLA-4) is most commonly implicated with around 5% of patients showing suggestive radiologic findings of sarcoidosis after treatment with ipilimumab (18). Patients may also have parenchymal findings but rarely have concomitant abdominal lymphadenopathy. Organ involvement is typically avid on fluorodeoxyglucose PET imaging and therefore difficult to distinguish from malignancy. Our patient also presented with splenic involvement, which can occur in a sarcoid-like reaction since granulomatous infiltration of the spleen can present as homogenous uptake on FDG/PET or small intrasplenic lesions (19, 20). Sarcoid-like reactions typically occur 3–6 months after treatment initiation, and it is often asymptomatic although there may be accompanying dyspnea, cough, skin manifestations, or other systemic symptoms (18, 21, 22). Given the difficulty in differentiating sarcoid-like reaction from malignancy or infectious causes based on imaging, biopsy is often performed. Similarly to sarcoidosis, a biopsy typically reveals non-caseating epithelioid and giant cell granulomas as seen in our patient. In a review of 18 reported cases, ICI was halted in 14 patients and 9 were treated with prednisone. Resolution occurred in all patients except for two who remained stable without treatment (23). While it usually has a benign course, clinicians should be aware of the possibility of sarcoid-like reactions following immunotherapy to prevent misinterpretation of imaging studies as treatment failure or progression.

While a sarcoid-like reaction was considered, the diffuse abdominal lymphadenopathy, persistent fevers, splenic calcified granulomata, and significant farm exposure raised concerns for disseminated histoplasmosis. Previous CT imaging from over 10 years prior showed evidence of splenic granulomata, indicating the possibility for reactivation. Despite the negative blood histoplasma antigen testing, there was a high enough index of suspicion to empirically treat given the limitations of this assay. Notably, a multicenter trial of the enzyme immunoassay-based test for the histoplasma antigen found a sensitivity of 91.8% for disseminated histoplasmosis, but the sensitivity was much lower (30%) for subacute histoplasmosis (24). Testing for the antigen from blood and urine can increase sensitivity (25). His histoplasma antibody was negative as well although his antibody response may have been inhibited secondary to his recent lymphoma and lymphoma treatment. Serious infections are not a common irAE during treatment with ipilimumab and nivolumab. While one study reported a 7.3% incidence of serious infection in patients with melanoma treated with immunotherapy, the main risk factor found in this study was treatment with corticosteroids or infliximab. The risk of serious infection was only 2% in those treated with immunotherapy without corticosteroids or infliximab and mainly consisted of bacterial infections (26). In fact, there is evidence that the PD-1/PD-L1 pathway is critical to fungal immune evasion, and PD-1 inhibition was shown to drastically improve the host immune response in mouse models of histoplasmosis (27).

In this case, the patient was treated empirically for disseminated histoplasmosis given his exposure history and persistent fevers with improvement in his lymphadenopathy on PET scan 2 months after initiating treatment. Given concern for an irAE, ipilimumab and nivolumab were discontinued, but the patient never required steroids given lack of pulmonary symptoms and normal spirometry. Fortunately, even those patients requiring discontinuation of immunotherapy seem to have improved outcomes compared with first-line tyrosine kinase inhibitors. The CheckMate 214 study compared previously untreated patients with RCC randomized to nivolumab and ipilimumab for 4 cycles followed by nivolumab versus treatment with oral sunitinib (3). Treatment-free survival (TFS) was compared between patients who discontinued treatment, and the median TFS was found to favor immunotherapy (28). Our patient did not require additional treatment until progression of a chest wall mass requiring radiation therapy almost 6 months after discontinuation of immunotherapy, and he has not required further systemic therapy.

CONCLUSION

With the expanding use of ICIs across multiple solid tumors, many questions remain regarding their optimal use, toxicity management, duration of treatment, and personalized biomarkers, among others. Our case sheds light on the challenges physicians encounter managing new onset lymphadenopathy on

ICI therapy considering the wide range of potential etiologies, and it highlights the importance of biopsy of indeterminate lesions as well as the multidisciplinary management of suspected ICI toxicity. In addition, our case illustrates the limitations of diagnostic testing for invasive fungal infections and the importance of clinical suspicion based on environmental exposures and characteristic radiologic findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MS, YG, and RA were responsible for the concept of the paper and wrote the manuscript. YG and RA treated the patient. ZM and EB interpreted the pathology and provided pathology images for the manuscript. All authors contributed to the article and approved the submitted version.

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Nivolumab drug holiday in patients treated for metastatic renal cell carcinoma: A real-world, single-centre experience

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Introduction: Immunotherapy with nivolumab (a monoclonal antibody that targets the programmed cell death protein 1, PD1) has become the standard treatment for patients with metastatic renal cell carcinoma (mRCC) after progression to single-agent tyrosine kinase inhibitors. However, the optimal duration of immunotherapy in this setting has not yet been established.

Patients and methods: We retrospectively reviewed all patients treated with nivolumab at our institution from January 2014 to December 2021 and identified those who discontinued treatment for reasons other than disease progression (PD). We then associated progression-free survival (PFS) and overall survival following treatment cessation with baseline clinical data.

Results: Fourteen patients were found to have discontinued treatment. Four patients (28.6%) ceased treatment due to G3/G4 toxicities, whereas the remaining ten (71.4%) opted to discontinue treatment in agreement with their referring clinicians. The median duration of the initial treatment with nivolumab was 21.7 months (7.5-37.3); during treatment, two patients (14.3%) achieved stable disease as the best response, and the remaining twelve (85.7%) a partial response. At a median follow-up time of 24.2 months after treatment discontinuation, 7 patients (50%) were still progression-free. The median PFS from the date of discontinuation was 19.8 months (15.2 - not reached); a radiological objective response according to RECIST and treatment duration of more than 12 months were associated with a longer PFS. Three patients were re-treated with Nivolumab after disease progression, all of whom achieved subsequent radiological stability.

Conclusion: In our experience, the majority of patients who discontinued treatment in the absence of PD were still progression-free more than 18 months after discontinuation. Patients whose initial treatment duration was less than 12 months or who did not achieve a radiological objective response had a greater risk of progression. Immunotherapy rechallenge is safe and seems capable of achieving disease control.

KEYWORDS

mRCC, renal cell carcinoma, anti-PD1, immunotherapy, rechallenge

Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults and accounts for 3-5% of new cancer diagnoses each year (1, 2). Nowadays, incidental early-stage RCC diagnoses account for the majority of new cases, but a significant proportion of patients with localised disease will still develop metastases at some point in time (3).

In recent years, immunotherapy in the form of immune checkpoint inhibitors (ICIs) has revolutionised the treatment of metastatic RCC.

Nivolumab, an ICI that targets the programmed cell-death protein 1 (PD1), has become the standard treatment for patients with mRCC following progression to single-agent tyrosine kinase inhibitors (TKI) (4). In combination with cabozantinib (a TKI) or ipilimumab (an ICI that targets the anti-Cytotoxic T-Lymphocyte Antigen 4), it is considered to be one of the standard treatments in previously untreated patients (5–7).

However, the maximum duration of treatment differed in those trials. In the 2015 Checkmate 025 trial (nivolumab vs. everolimus for pre-treated mRCC), the first trial that paved the way for nivolumab in the management of RCC, treatment continued until disease progression or the development of treatment-limiting toxicities (4). In the 2018 Checkmate 214 trial (nivolumab plus ipilimumab as first-line treatment), treatment with nivolumab was initially planned to continue until disease progression or the development of toxicities, but a subsequent amendment allowed the patient to discontinue therapy after two years (5, 8). Finally, in the 2021 Checkmate 9ER trial (nivolumab plus cabozantinib as first-line therapy), treatment with nivolumab had a maximum duration of two years from the start of treatment (6).

The reason for limiting the maximum duration of immunotherapy treatment is the growing body of evidence indicating that the disease's clinical control is often long-lasting and may be maintained even after therapy is discontinued. In fact, due to their unique mechanism of action, ICIs are capable of achieving long-term disease control in many solid malignancies, even after treatment discontinuation or interruption (9–12). Therefore, prolonged and ongoing treatment may not always be necessary for all patients.

Data from retrospective analyses indicated that treatment interruption after a certain number of cycles could be safe for selected patients (11–13). Moreover, other studies demonstrate the feasibility of presenting a rechallenge with ICIs in the event of disease progression following prior immunotherapy (14, 15).

A patient-tailored "stop and go" approach could be an alternative option for selected patients in order to reduce overtreatment, limit the occurrence of treatment-related toxicities, and improve the possible financial toxicity of those therapies without compromising the treatment's oncological results (in terms of clinical benefit and preservation of quality of life).

This paper presents a retrospective analysis of patients treated with nivolumab at our institution, who opted to discontinue treatment in the absence of disease progression.

Patients and methods.

We retrospectively reviewed all patients treated with nivolumab at our institution from January 2014 to December 2021 and identified those who discontinued treatment for reasons other than disease progression. Clinical data were extracted from electronic patient records.

Inclusion criteria included a histological diagnosis of RCC, previous treatment with nivolumab interrupted in the absence of PD, and the availability of all necessary data.

From electronic patient charts, we collected baseline clinical data, the reason for treatment discontinuation, the treatment's oncological outcome (including duration of initial treatment, best radiological response, development of immune-related toxicities, date of disease progression, and date of death or last follow-up), and data about subsequent treatments administered after disease progression. Adverse events were graded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0; radiological response was

defined using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.

Treatment duration was defined as the time between the first and last dose of nivolumab. Progression-free survival (PFS) was calculated using the Kaplan-Meier method from the date of treatment interruption to the date of disease progression or death (whichever occurred first); progression-free survival was censored at the last patient follow-up visit without progression. Overall survival was calculated from the date of drug interruption to the date of death from any cause. For patients re-treated with nivolumab after disease progression, PFS for the second course of immunotherapy was calculated from the beginning of the second course until the occurrence of new disease progression.

Key metrics were summarised by means of descriptive statistics. Patient PFS and OS were compared using the log-rank test and Cox's proportional hazards method (when applicable). We performed univariate and multivariate analyses to determine the association between baseline characteristics and PFS from the time of treatment discontinuation; the covariates that showed any association with the oncological outcome with a p value of at least less than 0.1 in the univariate analyses were included in the multivariate analysis. Results were classified as statistically significant if their p-values were < 0.05. All statistical analyses were performed with "R" v4.0.5 and the "survival" package v2.44-1.1.

At the time of their first visit to our institution, all patients gave their written consent for the use of their clinical data for scientific purposes. The study was conducted in accordance with the Declaration of Helsinki. Data collection was approved by the local Ethical Committee.

Results

Patient characteristics

Fourteen patients were found to have discontinued treatment for reasons other than disease progression. The median age was 77.7 years (range: 42.3-82.1 years). Eleven patients had been diagnosed with clear cell RCC (78.6%), one with papillary RCC, one with chromophobe RCC and one with RCC not otherwise specified. Twelve patients were treated with nivolumab in the second-line setting, while two patients were treated in the third-line. All but one patient had received nephrectomy prior to treatment. All patients were in good clinical condition at the start of Nivolumab treatment (ECOG PS of 0 or 1); 5 patients were classified as belonging to the good risk class according to IMDC criteria, while the remaining 9 patients were classified in the intermediate risk class; none of the patients were considered to be at poor risk. Patient clinical characteristics are summarised in Table 1.

Initial treatment details

The median duration of initial treatment with nivolumab was 21.7 months (7.5-37.3). During treatment, two patients (14.3%) achieved stable disease as the best radiological response, while the remaining twelve patients (85.7%) achieved a partial response. Twelve patients (85.7%) developed immune-related adverse events of any grade during therapy, requiring at least a brief interruption of nivolumab or treatment with systemic corticosteroids; four patients reported the onset of grade 3/4 toxicities (one grade 3 colitis, two grade 3 myocarditis and three grade 3 hypertransaminasemia). Data on treatment outcomes are reported in Table 2.

Cause of discontinuation, PFS from interruption and factors associated with PFS

Ten patients (71.4%) opted to discontinue treatment in agreement with their referring clinicians; however, for 5 of these patients (50%),

TABLE 1 Patient clinical characteristics.

Characteristics	Number of patients
Gender	
Male	10 (71.4%)
Female	4 (28.6%)
Age (years)	
Mean (range)	77.6 (42.3-82.1)
>70 years (%)	12 (85.7%)
Histology	
Clear Cell	11 (78.6%)
Other histologies	3 (21.4%)
Previous nephrectomy	
Yes	13 (92.9%)
No	1 (7.1%)
Metastases locations (number of patients)	
Lymph nodes	7 (50%)
Bone	3 (21.4%)
Liver	3 (21.4%)
Lung	10 (71.4%)
Small tissue	3 (21.4%)
Adrenal	2 (14.3%)
Others	3 (21.4%)
Performance Status (ECOG)	
0	4 (28.6%)
1	10 (71.4%)
IMDC risk classification	
Good	5 (35.7%)
Intermediate	9 (64.3%)
Poor	0 (0%)
Setting	
II line	12 (85.7%)
III line	2 (14.3%)

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TABLE 2 Details of patient baseline characteristics, initial treatment, therapeutic pause and post-progression course.

	Histology	Age (y)	PS	Tx duration (m)	BR	Reason for interruption	iRAE during initial tx	Drug holiday (m)	PD	Tx at PD	BR at rechallenge	Duration of rechallenge
1	RCC NOS	80.2	0	8.9	PR	Decision	Skin reaction (G2)	5.1	No	NA	NA	NA
2	Clear cell RCC	81	1	27.8	SD	Decision	None	15.2	Yes	Nivolumab	SD	12
3	Clear cell RCC	82.1	0	24.7	PR	Decision	None	26.3	No	NA	NA	NA
4	Clear cell RCC	80	1	24.3	PR	Decision	Skin reaction (G2)	16.1	Yes	SBRT	NA	NA
5	Clear cell RCC	75.9	0	21.4	PR	Decision	Uveitis (G2)	25	No	NA	NA	NA
6	Clear cell RCC	82	1	37.3	PR	Decision	Skin reaction (G2)	19.8	Yes	BSC	NA	NA
7	Clear cell RCC	53	0	12.9	PR	Decision	Arthralgia (G2)	18.4	No	NA	NA	NA
8	Clear cell RCC	74.6	0	22	PR	Decision	Pneumonia (G2)	37.1	No	NA	NA	NA
9	Papillary RCC	78.7	1	7.5	PR	irAE	Hypertransaminasemia (G3), hyperglycaemia/ diabetes (G2)	10	Yes	Nivolumab	SD	5
10	Clear cell RCC	77.6	1	22.7	PR	Decision	Skin reaction (G2)	35.7	No	NA	NA	NA
11	Clear cell RCC	42.3	0	8.7	SD	irAE	Hypertransaminasemia (G3)	5.6	Yes	Nivolumab	SD	4
12	Chromophobe RCC	74.3	1	32.7	PR	irAE	Colitis (G3)	13.5	Yes	Cabozantinib	PR	NA
13	Clear cell RCC	73	1	7.9	PR	irAE	Myocarditis (G3)	15.6	Yes	BSC	NA	NA
14	Clear cell RCC	77.8	1	11.2	PR	Decision	Skin reaction (G2)	5.7	No	NA	NA	NA

RCC, renal cell carcinoma; NOS, not otherwise specified; BR, best response; PR, partial response; SD, stable disease; PS, performance status (sec ECOG); Tx, treatment; BSC, best supportive care; iRAE, immuno-related adverse event; G2 or G3, grading per CTCAE; SBRT, sterotactic body radiation therapy; NA, Not Applicable.

the previous occurrence of low-grade (G1-G2) adverse events was an important factor in their decision. The other four patients (28.6%) discontinued treatment after developing G3/G4 toxicities.

At a median follow-up time of 24.2 months after treatment discontinuation, 7 patients (50%) were still progression-free. For 5 of the 7 patients who progressed, radiological progression was defined by the enlargement of known pre-existing lesions, and for the other 2, by the emergence of metastases at new sites (two new lesions in the liver and a brain metastasis, respectively).

The median PFS from the date of discontinuation until disease progression was 19.8 months (15.2 - not reached); the median overall survival was not reached, with just one patient having died by the time of data cut off. Data on the post-interruption outcomes are reported in Table 2; Figure 1.

At univariate analysis, stable disease as the best radiological response and a treatment duration of less than 12 months were associated with a worse PFS (Table 3); sex, IMDC risk group, performance status at the start of the interruption and the development of immune-related adverse events (irAEs) during treatment were not significantly associated with PFS (Table 3). The prognostic value of treatment duration and radiological response were maintained at multivariate analysis (Table 3).

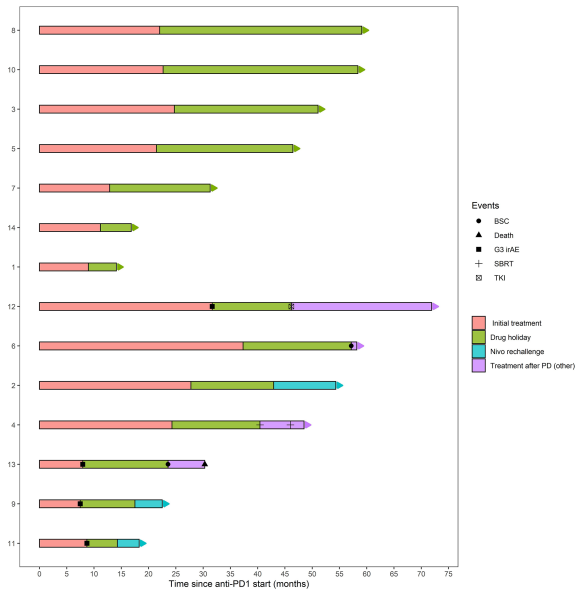

FIGURE 1
Duration of initial treatment, treatment free interval and subsequent therapies in patients with (below) or without (above) disease progression after nivolumab interruption. BSC, best supportive care; SBRT, stereotactic body radiation therapy.

Treatment after disease progression

After disease progression, two patients were considered ineligible for other oncological treatments due to their poor

TABLE 3 Univariate and multivariate analyses of characteristics associated with PFS after nivolumab discontinuation.

Characteristics	Univariate HR (95% CI)	p	Multivariate HR (95% CI)	p
Gender				
M vs. F	1.41 (0.27-7.4)	0.68		
Age (years)	0.97 (0.91 – 1)	0.46		
Duration of initial treatment				
> 12 vs. ≤ 12 months	0.12 (0.019-0.73)	**0.02**	0.06 (0.01-0.62)	**0.018**
IMDC risk group				
Interm. vs. Good	0.61 (0.13-2.8)	0.52		
Occurence of irAEs				
Yes vs. No	1.3 (0.15 - 11)	0.82		
Basal PS				
1 vs. 0	0.82 (0.16-4.3)	0.82		
Radiological BR				
SD vs. PR	7.9 (1.1-57)	**0.04**	18.1 (1.38-237)	**0.028**

HR, hazard ratio; M, male; F, female; BR, best response; PR, partial response; SD, stable disease; PS, performance status (sec ECOG); iRAE, immuno-related adverse event; IMDC, International Metastastic RCC Database Consortium.
Bold values are statistically significant values.

clinical condition and were, therefore, only treated with best supportive care. One patient, whose CT scan revealed an oligoprogressive disease, was successfully treated twice in succession with stereotactic ablative radiotherapy and has not yet begun additional systemic therapy.

Systemic therapy was initiated for the other four patients: due to the previous occurrence of immune-related colitis, one patient started third-line treatment with cabozantinib; the other three patients were re-treated with nivolumab. For two of these three patients, the cause for initial discontinuation was the emergence of an irAE (two grade 3 hypertransaminasemia) At the time of data cut off, the patients re-treated with nivolumab had been treated for 4, 5 and 12 months and are all progression-free; to date, no immune-related adverse event of any grade has been reported for either of them. Data on the treatments administered after disease progression and outcomes are reported in Table 2; Figure 1.

Discussion

Immunotherapy has drastically improved the prognosis and natural history of patients with advanced renal cell carcinoma. The role of immunotherapy has been enhanced with the publication of recent trials, and a combination treatment with an immune checkpoint inhibitor is currently considered to be the standard of care in the first-line setting (7). Nevertheless, the definition of the optimal immunotherapy treatment duration is a clinical need that is still unmet.

Despite the fact that in the first and older trials, treatment with immune checkpoint inhibitors was continued until disease progression or the development of severe toxicities, ICIs have been shown to achieve long-term disease control even in the event of interruption (for example, due to adverse events or decisions by physicians or patients) (11, 12). There is strong biological evidence to support the fact that for many patients, especially those who are able to achieve a dimensional response at the radiological assessment, the continuation of treatment until progression occurs is not always necessary (13).

Long-term follow-up analysis of clinical trials using ICIs in melanoma and NSCLC demonstrated that many patients maintain the therapeutic benefit long after the end of treatment (11, 12).

In the case of RCC, 27 patients with a response to nivolumab discontinued treatment in the Checkmate 025 trial and never received additional subsequent systemic therapy (with a median treatment-free interval of 12.7 months); 13 of these patients were still alive and free from disease progression at the last follow-up (10).

Many clinical trials are currently set for a maximum 2-year period of ICI treatment for all patients enrolled (6, 16). However, it is not clear whether this fixed duration is totally necessary or whether treatment could be discontinued earlier in selected patients (or should be continued for other patients, even after this arbitrary cut off).

In metastatic melanoma, a retrospective analysis of patients treated with anti-PD1 (pembrolizumab or nivolumab) for a median initial treatment duration of 12 months showed that the risk of relapse after treatment discontinuation was low, particularly in patients who achieved complete radiological response during treatment (13).

Conversely, in non-small cell lung cancer (NSCLC), a randomised trial revealed that a fixed duration of one year seems to be inferior, in terms of PFS and OS, to continuous treatment with nivolumab in the whole population (17).

Several authors have investigated the optimal duration and management of ICI treatment for RCC. In a recent phase II trial, 5 out of 12 patients (42%) who opted to discontinue nivolumab after achieving a radiological response within the first 6 months of treatment were progression-free one year after the discontinuation of treatment (18).

Ornstein et al. conducted a phase II trial to evaluate the outcomes of intermittent treatment with nivolumab in a similar setting; of five patients who opted to discontinue nivolumab after obtaining a radiological reduction of 10% in tumor size, only one patient had to restart treatment at a median follow-up of 48 weeks (19).

These small trials demonstrate that, for some patients, treatment interruption could be a viable option, but additional and larger studies are needed to increase the level of evidence and refine patient selection.

However, following the decision to discontinue treatment, another important unanswered question concerns the immunotherapy rechallenge's efficacy. Retrospective analysis in patients with other solid malignancies revealed an interesting response rate and a clinical benefit in patients re-treated with immunotherapy after disease progression (with the same ICI after a therapeutic pause or with a different ICI in the event of PD during treatment) (15, 20).

In a retrospective, multicentric analysis of renal cell carcinoma, Ravi et al. found a response rate of 23% with low incidence of severe adverse events in a cohort of 69 patients (50 of them discontinued initial treatment due to PD and 16 due to irAEs) who underwent anti-PD1/anti-PDL1 rechallenge treatment (14). The occurrence of grade 3-4 irAEs was reported by 18 patients (26%) during the first immunotherapy course and by 11 patients during the rechallenge, but only 3 of these patients had previous G3 toxicity during initial treatment (14).

The rechallenge strategy must be evaluated differently depending on whether the decision to discontinue therapy was due to the occurrence of toxicities or due to the patients' or physicians' preferences, as opposed to the progression of disease during treatment. Unfortunately, many studies, such as the abovementioned ones, did not distinguish between patients whose disease was under control or progressing when they discontinued treatment. These clinical situations are clearly distinct, and the results of re-treatment in one setting may not be applicable in another.

In fact, recent trials specifically designed for patients after progression or a lack of response to treatment with a single ICI are evaluating the intensification strategy using combination treatment (TKI plus anti-PD1 or anti-PD1 plus another ICI) rather than a single ICI (21, 22).

The final important question concerns the rechallenge's toxicity profile. Many retrospective analyses demonstrated that, for patients who previously discontinued immunotherapy due to the occurrence of irAEs, these irAEs do not typically recur after the immunotherapy rechallenge's commencement. Moreover, irAEs are usually milder and more manageable during rechallenge (15, 23–25). Due to the retrospective nature of these studies, toxicity profile data must be interpreted with caution. In fact, selection bias is a significant limitation, and it is likely that the patients selected for a rechallenge were those who only experienced non-life-threatening, minor and transient adverse events (AEs) in the first course of therapy.

The majority of patients in our population who opted to discontinue treatment were safe and progression-free after more than one year from the start of the therapeutic break. As reported by other authors, the risk of progression was lower in patients who had been treated for more than 12 months and in patients who had previously achieved an objective radiological response. Re-treatment appeared to be safe for patients who had progressed; it is interesting to note that, despite the limitations of a short follow-up, no treatment-related adverse events were reported, in spite of the fact that two of the patients had initially discontinued treatment due to grade 3 toxicities (hypertransaminasemia). Accordingly, we decided not to re-treat the patient who had previously reported grade 3 colitis.

Our analysis has several limitations. Due to the retrospective design, there was a selection bias in the population, which consisted of patients with a very good clinical condition and good prognostic characteristics at baseline. The small sample size limited the possibility of finding prognostic and predictive indicators for a prolonged drug holiday period; this could explain why many well-established prognostic factors, such as the IMDC class and performance status, did not seem to be associated with this PFS. Finally, radiological evaluation was performed as per the clinician's decision, and radiological images were not re-examined.

Conclusion

In our experience, the discontinuation of nivolumab treatment in a cohort of highly selected patients seems to be safe and capable of sustaining the disease's long-term clinical control. Treatment duration of more than one year and the achievement of a radiological objective response were prognostic of longer progression-free survival from the date of treatment discontinuation. Rechallenge with nivolumab after the occurrence of progression seemed to be safe for the selected patients, including those patients who had previously reported the occurrence of certain toxicities.

More studies are urgently needed to determine the optimal duration and management of treatment with ICIs, especially given the ever increasing importance of immunotherapy. An improvement in the selection of patients who can safely discontinue treatment with ICIs could result in a dramatic improvement in treatment customisation and individualisation.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Available upon request. Requests to access these datasets should be directed to marco.maruzzo@iov.veneto.it.

Ethics statement

The studies involving human participants were reviewed and approved by Comitato Etico IOV IRCCS. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MM, DB, and VZ study design. EL, NC and AM data collection. MD, DB and FP data analysis. MD, DB and MM data interpretation and review. UB, DB and MM supervision. All authors: final review and approval of the final paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The cuproptosis-associated 13 gene signature as a robust predictor for outcome and response to immune- and targeted-therapies in clear cell renal cell carcinoma

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Cuproptosis, the newly identified form of regulatory cell death (RCD), results from mitochondrial proteotoxic stress mediated by copper and FDX1. Little is known about significances of cuproptosis in oncogenesis. Here we determined clinical implications of cuproptosis in clear cell renal cell carcinoma (ccRCC). Based on the correlation and survival analyses of cuproptosis-correlated genes in TCGA ccRCC cohort, we constructed a cuproptosis-associated 13 gene signature (CuAGS-13) score system. In both TCGA training and two validation cohorts, when patients were categorized into high- and low-risk groups according to a median score as the cutoff, the CuAGS-13 high-risk group was significantly associated with shorter overall survival (OS) and/or progression-free survival (PFS) independently (P<0.001 for all). The CuAGS-13 score assessment could also predict recurrence and recurrence-free survival of patients at stage I – III with a high accuracy, which outperformed the ccAccB/ClearCode34 model, a well-established molecular predictor for ccRCC prognosis. Moreover, patients treated with immune checkpoint inhibitors (ICIs) acquired complete/partial remissions up to 3-time higher coupled with significantly longer PFS in the CuAGS-13 low- than high-risk groups in both training and validation cohorts of ccRCCs (7.2 – 14.1 *vs.* 2.1 – 3.0 months, P<0.001). The combination of ICI with anti-angiogenic agent Bevacizumab doubled remission rates in CuAGS-13 high-risk patients while did not improve the efficacy in the low-risk group. Further analyses showed a positive correlation between CuAGS-13 and TIDE scores. We also observed that the CuAGS-13 score assessment accurately predicted patient response to Sunitinib, and higher remission rates in the low-risk group led to longer PFS (Low- *vs.* high-risk, 13.9 *vs.* 5.8 months, P = 5.0e-12). Taken together, the CuAGS-13 score assessment serves as a robust predictor for survival,

recurrence, and response to ICIs, ICI plus anti-angiogenic drugs and Sunitinib in ccRCC patients, which significantly improves patient stratifications for precision medicine of ccRCC.

KEYWORDS

ccRCC, cuproptosis, immunotherapy, immune checkpoint inhibitors, prognosis, targeted therapy

Introduction

Clear cell renal cell carcinoma (ccRCC), derived from the epithelial cells in the nephron, is the predominant subtype of renal cell carcinoma (RCC) (up to 80% of all RCCs), and characterized by the inactivation of the *von Hippel Lindau (VHL)* gene and subsequent dysregulation of hypoxia-inducible factor (HIF)-responsive genes (1–4). ccRCC incidence has increased over the past decades worldwide (3), while fortunately, most patients are diagnosed at early stages with localized disease, and thus successfully resected (2). However, approximately 30% of these patients will undergo recurrence post-operation (2). Traditionally, patient clinicopathological features are applied to evaluate recurrence risk and to predict prognosis (5). More recently, efforts have been made to identify molecular biomarkers for reliable outcome prediction of ccRCC (5). Towards this purpose, several studies developed multigene expression signatures, and these signatures, either alone or together with the traditional stratification system, were shown to improve ccRCC prognostication (5–11). Despite so, molecular and clinicopathological parameters are still far from accurately predicting patient outcomes. It is thus demanding tasks to further develop new biomarkers or molecular tools for ccRCC prognosis and personalized interventions.

ccRCC is intrinsically insensitive to chemotherapy, and therefore, other treatment strategies have been applied (12). For instance, interleukin 2 (IL2), as an immunotherapeutic agent, has been widely used for metastatic ccRCC (mccRCC) since decades ago, which achieved complete and durable responses in a fraction of patients (13, 14). However, severe side-effects significantly restricted the application of IL2 treatment (15). More recently, boosting anti-cancer immune response using immune checkpoint inhibitors (ICIs) have revolutionized the cancer therapy (15, 16). By targeting immune checkpoint proteins PD-1/PDL-1 and/or CTLA4, the ICI strategy shows clinical benefits in various cancer types. Similarly, this approach has been successful in the treatment of localized ccRCC as adjuvant therapy after nephrectomy and mccRCC. However, response rates for ccRCC are in general less than 50% (15, 17). The combined treatment of ICIs with targeted therapeutic drugs such as Bevacizumab may improve efficacy (18–20). ccRCC exhibits unique immunological features, and high CD8 T infiltration correlates with poor prognosis, which contrasts with favorable outcomes observed in other cancer types (16, 21). In addition, tumor mutation burden (TMB) predicts ICI response in many solid tumors, but not in ccRCC (22, 23). PBRM1 mutations and expression of human endogenous retroviruses (HERVs) were shown to be associated with response in ccRCC by some studies but could not be validated in other reports (22, 24–27). More recently, other biomarkers have been developed to predict patient response to ICIs (21, 28). Thus, identifying reliable predictors for ICI response in ccRCC remains unmet demands. It is also poorly defined which patients will benefit more from the combined therapy of ICIs with Bevacizumab, which calls for further investigations.

In addition, Sunitinib, an inhibitor of multiple tyrosine-kinase receptors, was approved by FDA for the first line treatment of ccRCC in 2006 (29). Most patients benefit from the treatment with longer progression-free survival (PFS), but approximately 1/3 of ccRCCs exhibit intrinsic resistance to Sunitinib (12). Distinguishing Sunitinib responders from non-responders is clinically important.

One of the cancer hallmarks is an increased capacity for survival (30). Evading apoptosis is the well-defined mechanism for cancer cells to evade death fate (30). There exist other forms of regulated cell death (RCD), such as ferroptosis, paraptosis and pyroptosis, and they similarly play a part in modulating cancer cell survival (31). More recently, a copper-dependent cell death, so-called cuproptosis, was identified by Tsvetkov et al. (32). Mechanistically, the reductase FDX1 and copper induce the lipoylation and aggregation of mitochondrial enzymes responsible for the tricarboxylic acid (TCA) cycle, and promote Fe-S cluster protein degradation, thereby leading to proteotoxic stress and cell death (32). It is currently unclear whether cuproptosis contributes to the ccRCC pathogenesis and has any clinical implications in ccRCC managements. The present study is designed to address these issues. By analyzing TCGA and other datasets, we identified the cuproptosis-associated 13 gene signature (CuAGS-13) as a predictor for patient survival, recurrence and response to ICI, Bevacizumab and Sunitinib treatments in ccRCC.

Materials and methods

Data collection and processing of ccRCC tumors

The TCGA cohort of ccRCCs included 525 tumor samples with survival information available and 72 nontumorous adjacent renal tissues (11). Transcriptome, mutation, copy number variations (CNAs) and clinical-pathological data were downloaded from https://gdc.cancer.gov/. One hundred and one patients with ccRCC were in the E-MTAB-1980 cohort (33), and RNA array and clinical information were downloaded from http://www.ebi.ac.uk. The ICGC-RECA-EU cohort included 91 ccRCC patients and their clinical and RNA sequencing data were downloaded from https://dcc.icgc.org/. For RNA sequencing data, mRNA abundances were expressed as Transcripts Per Million (TPM). Microarray data of patient-derived xenografts (PDX) models in GSE64052 were downloaded from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/). For array results (determined by 4×44K v2 microarray kit) from the E-MTAB-1980 cohort and GSE64052, probe-set values were used to quantify mRNA levels. ccRCC patients receiving ICIs, ICIs plus Bevacizumab, and Sunitinib treatments were contained in IMmotion150 (34, 35), CheckMate025 (23, 24) and IMmotion151 trials (18, 36). No ethics approval is required for the present study.

Identification of cuproptosis-associated genes using weighted gene co-expression network analysis

For 525 tumors and 72 adjacent non-cancerous renal tissues in the TCGA ccRCC cohort, the single sample gene set enrichment (ssGSEA) analysis was carried out to calculate the cuproptosis ssGSEA score in each sample according to expression levels of 10 cuproptosis genes (*FDX1*, *LIAS*, *LIPT1*, *DLD*, *DLAT*, *PDHA1*, *PDHB*, *MTF1*, *GLS* and *CDKN2A*). The enrichment statistic (ES) value in each sample (ssGSEA score) was calculated using GSVA package based on standardized mRNA levels [log2(TPM+1)] of each sample. WGCNA analyses were then performed to establish a co-expression network based on the cuproptosis ssGSEA score (Figure S1). Towards this end, hierarchical clustering by average link first detected outliner samples for exclusion (Figure S1A) and the Pearson's correlation matrices were then applied for all pair-wise genes followed by the construction of a weighted adjacency matrix. The soft-thresholding parameter or β value, which highlights strong correlations while penalizes weak correlations between genes, was set at 6 (scale free R^2 = 0.80) for a scale-free net-work based on the scale independence and mean connectivity (Figure S1B). The generated adjacency matrix was further transformed into the topological overlap matrix (TOM). All genes were categorized into co-expression modules according to TOM-based dissimilarity using an average linkage hierarchical clustering method. The first principal component of expression matrix is set as module eigengenes (Figure S1C). A total of 27 modules were finally identified (Figure S1C). Among these modules, brown and magenta ones were highly correlated with the cuproptosis score (Figure 2A). In addition, tumor and immune scores were integrated into the analysis of the relationship between cuproptosis score and tumor/immune scores (Figure 2A).

Construction of the CuAGS-13 risk score

Using the threshold for module membership correlation >0.5 and gene significance Cor >0.2, we acquired a total of 872 genes, among which 771 genes (in brown modules) correlated with while 101 genes (in magenta module) anti-correlated with the cuproptosis score. The impact of these 872 gene levels on progression-free survival (PFS) was evaluated using univariate COX regression and K-M analyses and 315 genes were selected for further analysis by the least absolute shrinkage and selector operation (LASSO) regression. Thirteen genes were finally acquired as the cuproptosis-associated gene signature or CuAGS-13 after verification by the Cox proportional-hazards model. We calculated CuAGS-13 score in each sample based on the following formula:

Score = Σ βi × RNAi, where βi is the coefficient of the i-th gene in multivariable Cox regression analysis, and RNAi is RNA expression level of gene i. Patients were divided into the high- and the low-risk groups using the median score as a cut-off. Differences in survival (OS, PFS and RFS), recurrence, and response to ICIs or Sunitinib between the high- and low-risk groups were analyzed using packages of the R software. The accuracy of the prediction is evaluated using the ROC curve. For comparison with the ccA/ccB/ClearCode34 model, the classification of the TCGA cohort was directly from published data by Brook et al. (7) and Buttner et al. (8).

Expression differences in CuAGS-13-containing 13 genes were compared between ccRCC tumors and non-tumorous adjacent renal tissues in the TCGA cohort. For RNA expression, log2(TPM+1) based on RNA sequencing data was form https://gdc.cancer.gov/ as stated above. Protein expression data was obtained from Clinical Proteomic Tumor Analysis Consortium (http://ualcan.path.uab.edu/index.html).

Development of a predictive nomogram for survival and recurrence

Cox regression analysis was performed to determine the impact of the CuAGS-13 score and clinical variables on survival and recurrence. Thereafter, based on multivariate Cox regression analysis results, we constructed a predictive nomogram that included CuAGS-13 score, age, grade and stage to predict 1-, 3-,

and 5-year survival (OS, PFS and/or RFS) and recurrence. Predicted survival of the nomogram against observed ones was plotted using the calibration curve. All nomograms and assessments of their predicative powers were made using R package regplot.

TIDE score analysis for response to ICIs

TIDE score is calculated based on myeloid-derived suppressor cell (MDSC), macrophage M2, T cell Dysfunction and Exclusion (37). TCGA ccRCC TIDE score was directly downloaded from http://tide.dfci.harvard.edu/. TIDE score for ccRCC cohort treated with Nivolumab was calculated online at http://tide.dfci.harvard.edu/. mRNA expression was standardized by using the all sample average expression as the normalization control prior to TIDE score analysis.

Gene set enrichment analysis

GSEA for KEGG (GSEA-KEGG) and Hallmark (GSEA-Hallmark) pathways (version 4.2.1 www.broadinstitute.org/gsea) was carried out to determine CuAGS-13 score-related signaling enrichments. Adjusted $P < 0.05$ and FDR <0.25 were defined as the activation or inhibition of signaling pathways.

Statistical analysis

All statistical analyses were carried out using R package version 4.0.5. Wilcox and K-W sum tests were used for analysis of differences between two groups and among multi groups, respectively. Spearman's Rank-Order Correlation coefficient was applied to determine correlation coefficients r between two variables. Survival analyses were made using log-rank test. The Survival and Survminer packages were employed to draw Kaplan–Meier survival curves for visualization of OS, PFS and RFS. Univariate and multivariate Cox regression analyses were used to determine the effect (HR and 95% CI) of various quantitative predictor variables on OS, PFS or RFS. Time-dependent ROCs and AUCs were made using Rpackage timeROC. $P < 0.05$ were considered as statistically significant.

Results

Construction of a cuproptosis-associated gene signature in the TCGA cohort of ccRCC

Ten factors, which include FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, and CDKN2A, have been identified to participate in the cuproptosis process (32) (Figure 1A). Among these factors, FDX1 functions as a key player to drive cuproptosis by reducing Cu^{++} to Cu^{+} (32) (Figure 1A). Because it is currently unclear which roles cuproptosis has in ccRCC pathogenesis, we first sought to determine whether these 10 molecules were associated with patient survival but failed to establish a satisfactory model in the TCGA cohort of ccRCC (Supplementary figures 2 and 3). We then made ssGSEA analysis to calculate the cuproptosis score in each sample based on the expression of 10 genes above, followed by the weighted gene co-expression network analysis (WGCNA) to look for cuproptosis-correlated genes (Figure 1B). By doing so, we identified that the cuproptosis score was (i) significantly correlated with 771 while anti-correlated with 101 genes; (ii) negatively associated with oncogenesis, indicating a tumor suppressive role of cuproptosis; and (iii) significantly correlated with immuneEstimate scores (Figure 2A). COX and LASSO regression analyses were then carried out to assess the impact of these 872 genes on patient progression-free survival (PFS) (Figures 2A–C). We finally acquired 13 genes as the cuproptosis-associated 13 gene signature, which we named as CuAGS-13. These 13 genes include TMEM214, CCM2, P3H4, FDX1, CDC42BPG, C11orf52, GNG7, PAQR5, ENAM, WDR72, SDR42E1, BSPRY and KDF1. The cuproptosis score was correlated negatively with the expression of TMEM214, CCM2 and P3H4, while positively with the rest of them. TMEM214, CCM2 and P3H4 expression was significantly higher in tumors than in their normal counterpart tissues (Figure 2D). In contrast, the expression of the rest 10 genes was dramatically downregulated in tumors (Figure 2D). Further CPTAC analyses of their protein expression (9 of 13 protein expression data available) showed that differences in protein levels between normal and tumors were largely similar to RNA expression trends (Figure S4). Each of these 13 factors was significantly associated with PFS when patients were divided into high and low categories using a median value as the cutoff (Figure 2E). In addition, the CuAGS-13 score was significantly associated with multi clinical-pathological variables including age, gender, grade, stage, metastasis and white cells in the TCGA ccRCC cohort (Table S1).

The CuAGS-13 score for survival prediction in ccRCC

We then sought to determine impacts of the CuAGS-13 score on OS and PFS in 525 ccRCC patients from the TCGA dataset as a training cohort (Table S1). According to the CuAGS-13 score in ccRCC tumors, patients were categorized into high- and low-risk groups using the median score value as a cut-off (> and ≤ median score, respectively). A Kaplan-Meier analysis revealed that patients in the high-risk group had significantly shorter OS and PFS (P<1e-11 and 1e- 20, respectively)

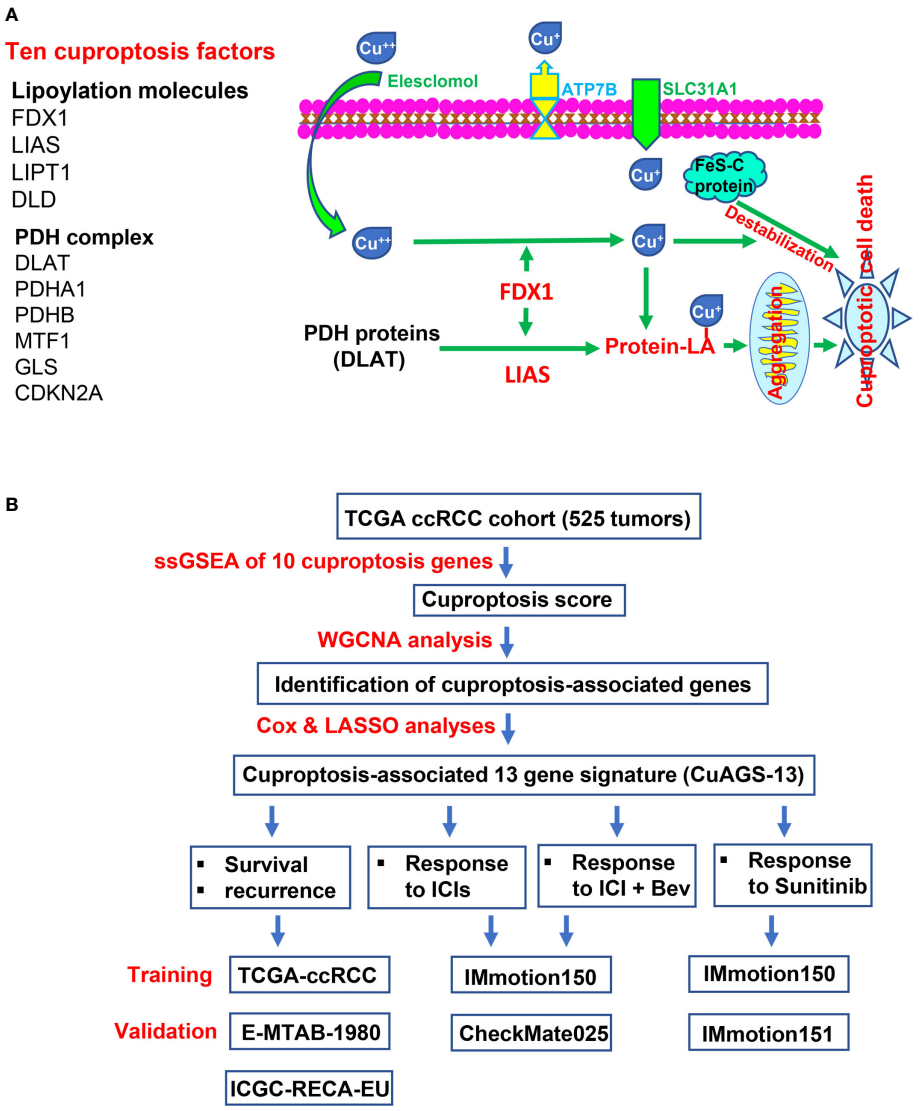

FIGURE 1
The Cuproptosis pathway and study workflow. **(A)** Left panel: Ten factors involved in cuproptosis. Right panel: The cuproptosis signaling pathway. Extracellular copper Cu^{++} enters cells by binding to copper chelators and elesclomol serves as the most efficient Cu^{++} transporter. The reductase FDX1 reduces Cu^{++} to Cu^{+}, a more toxic form, while lipoyl synthase (LIAS) catalyzes lipoylation of the pyruvate dehydrogenase (PDH) complex proteins including dihydrolipoamide S-acetyltransferase (DLAT) and others. Cu^{+} and lipoylation promote the protein aggregation. DLAT is one of the key enzymes participating in the *tricarboxylic acid* cycle, and its aggregation results in mitochondrial proteotoxic stress and subsequent cuproptotic cell death. Moreover, FDX1 and Cu^{+} induce the destabilization of Fe–S cluster proteins, further facilitating cuproptosis. Additionally, SLC31A1 and ATP7B function as the Cu^{+} importer and exporter, respectively, and regulate cuproptosis by controlling intracellular Cu^{+} concentrations. **(B)** The schematic workflow of the present study.

(Figure 3A). The risk score exhibited a high accuracy in predicting 1-, 3- and 5-year survival, as assessed by a time-dependent Receiver Operator Characteristic (ROC) curve (Figure 3B). Univariate COX regression survival analyses were further performed by including patient age, gender, stage, grade, and white cells together with the CuAGS-13 model. As shown in Figures 3C, D, stage, grade and CuAGS-13 score (high-risk) were all significantly associated with shorter OS and PFS, while White cells were associated with longer PFS without affecting OS, and female patients had longer PFS. Age was associated with shorter OS but not PFS. Multivariate analyses revealed that stage, grade and CuAGS-13 score (high-risk) were all independent prognostic factors for shorter OS and PFS, while age remained as a variable associated with shorter OS (Figure 3C). Based the results above, we established a prognostic nomogram composed of CuAGS-13 score, age, stage, and grade, which showed a highly

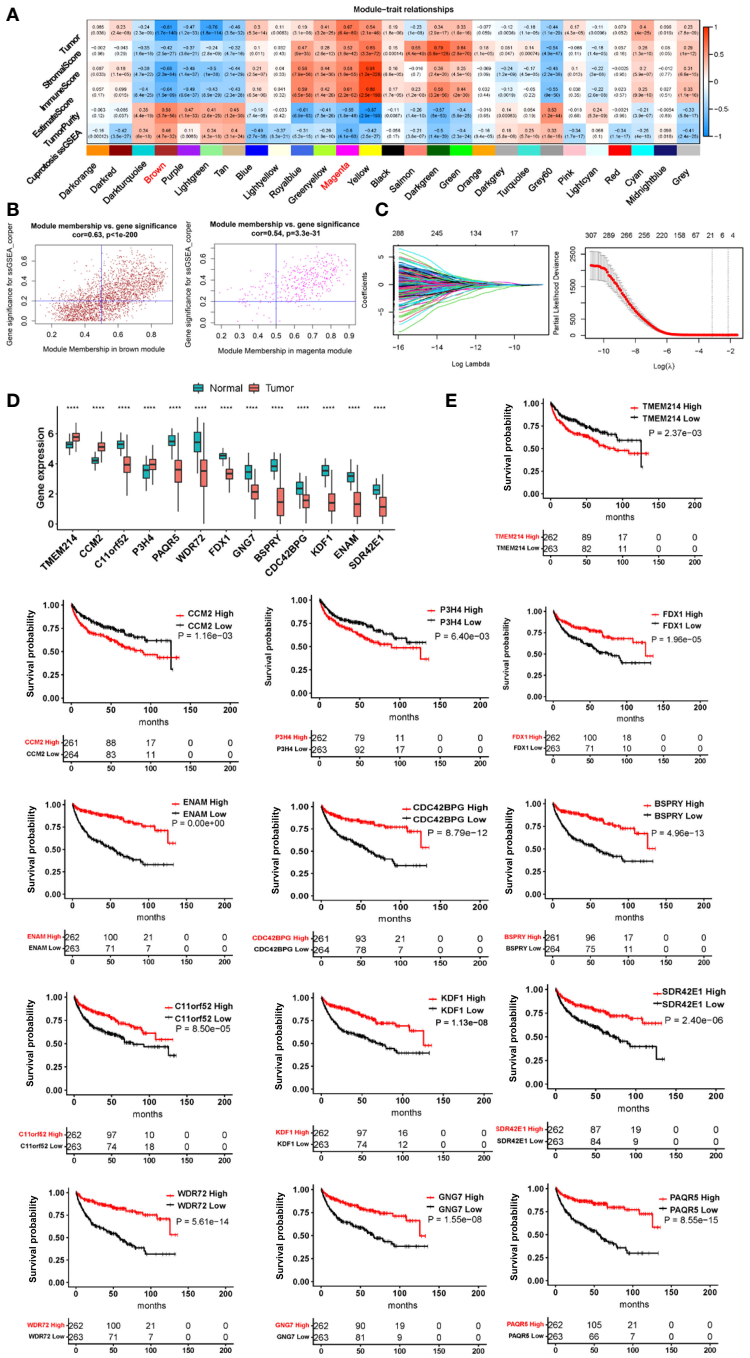

FIGURE 2
The construction of the cuproptosis-associated 13 gene signature (CuAGS-13) for ccRCC prognosis. **(A)** Left panel: Gene modules correlated with cuproptosis factors as determined using Weighted gene co-expression network analysis (WGCNA) and Pearson's co-efficiency analysis. **(B)** Scatter plot of module eigengenes in the MEBROWN (left) and MEMANGE (right) modules from **(A)**. The genes in the upper right are selected for further analyses. **(C)** Construction of the cuproptosis-associated 13 gene signature (CuAGS-13) for progression-free survival (PFS) prediction in ccRCC. Top panel: LASSO coefficient profiles of the CuAGS associated with PFS. Bottom panel: Plots of the cross-validation error rates. Each red dot represents a lambda value with its error bar (the confidence interval for the cross-validated error rate). The analysis identified 13 cuproptosis-associated genes most relevant to PFS. **(D)** Differences in the CuAGS-13 expression between ccRCC tumors and their non-tumorous adjacent renal tissues in the TCGA cohort. **(E)** Kaplan–Meier survival analysis showing the impact of each gene contained in CuAGS-13 on PFS in the TCGA ccRCC cohort. Patients are divided into high and low groups based on the expression of each gene in tumors using a median value as the cutoff. ****p < 0.0001.

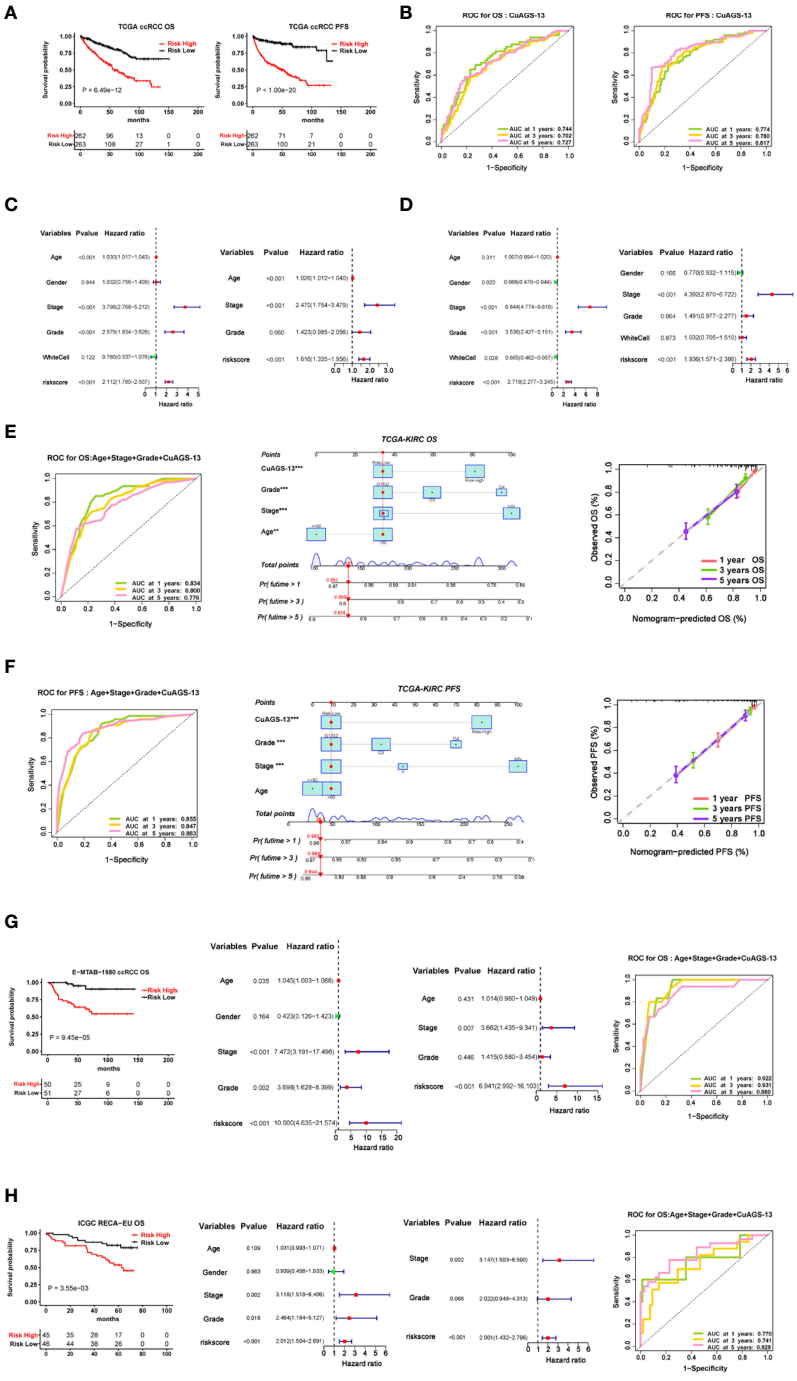

FIGURE 3
The cuproptosis-associated 13 gene signature (CuAGS-13) model for ccRCC survival prediction. **(A)** Kaplan–Meier survival analysis showing the significant association of the CuGAS-13 score with OS and PFS in the TCGA ccRCC cohort. Patients were classified into high- and low-risk groups based on the CuGAS-13 score using a median value as the cutoff. **(B)** The ROC curve showing a high accuracy in predicting 1-, 3- and 5-year OS and PFS using the CuGAS-13 model. **(C)** and **(D)** Univariate and multivariate Cox regression analyses of OS and PFS in ccRCC, respectively. **(E)** and **(F)** The nomogram composed of CuAGS-13 model, age, grade and stage for predicting 1-, 3- and 5-year OS and PFS, respectively. **(G)** The validation of the CuGAS-13 model for the prediction of OS in the EMBA-1980 cohort of ccRCC. **(H)** The validation of the CuGAS-13 model for the prediction of OS in the ICGC-RECA-EU cohort of ccRCC.

accurate estimation of survival possibilities at 1, 3 and 5 years (Figures 3E, F).

To confirm the findings in the TCGA ccRCC, we further assessed the effect of the CuAGS-13 score on survival of ccRCC patients from two other databases as validation cohorts. For the E-MTAB-1980 cohort of 101 patients (33), OS data were available, and their clinic-pathological characteristics were listed in Table S2. The CuAGS-13 score high-risk group had significantly shorter OS (P = 9.45e-10^5) and served as an independent prognostic factor as revealed by the multivariate Cox regression analysis (Figure 3G). The ROC curve further showed a robust power in predicting 1-, 3- and 5-year survival when the CuAGS-13 model was combined with age, grade and stage (Figure 3G). The ICGC-RECA-EU cohort included 91 ccRCC patients (Table S3) (https://dcc.icgc.org/) and our analysis results were very similar to those observed in E-MTAB-1980 cohort (Figure 3H). In those 91 patients, adjacent normal renal tissues from 45 were also analyzed for their expression profile, and the comparison in 13 gene expression between tumors and normal tissues showed largely same patterns as seen in the TCGA cohort except CDC42BPG (Figure S5).

The recurrence prediction of ccRCC patients by the CuAGS-13 model

Approximately 30% of localized ccRCC (I – III stages) will relapse after surgery, and it is clinically important to stratify those patients with a higher recurrence risk. We thus assessed the value of the CuAGS-13 score in recurrence prediction. Because the ccA/ccB/ClearCode34 molecular classifier has been successfully applied for such a purpose, we also made a comparison between it and our CuAGS-13 score system. We first analyzed all the patients at I -III stages in the TCGA cohort. The time-dependent ROC curves showed comparable sensitivity and specificity for predicting recurrence-free survival (RFS) with both models when combined with age, stage and grade (Figure 4A). However, these two models classified different patient groups (<50% of overlapping) as revealed by the Sankey diagram (Figure 4A right panel). Because patients at stages II and III are more unpredictable, we further analyzed these patients separately. As shown in Figure 4B, the CuAGS-13 score performed better, in all three time points. Moreover, we employed multivariate and co-occurrence index (C-index) analysis to predict recurrence in 377 patients at I – III stages. Recurrence occurred in 69 patients with time information available, and obtained results demonstrated that 56/69 (81.2%) and 44/69 (63.8%) were in the CuAGS-13 high-risk group and ccB subtype, respectively (P = 0.05) (Figure 4C). Patients at stage I and II-III were then analyzed separately. Twenty of 216 stage I patients underwent recurrence, and the analysis results by these two models did not differ significantly (Figure 4D), while the CuAGS-13 score significantly outperformed the ccA/ccB/ClearCode34 model in predicting recurrence for patients at stage II and III (CuAGS-13 score *vs.* ccA/ccB: 85.7% *vs.* 65.3%, P = 0.04) (Figure 4E); A Kaplan-Meier analysis also showed a better stratification of RFS in the stage II – III patient group using the CuAGS-13 score, whereas the ccA/ccB/ClearCode34 model failed to predict RFS in this group (Figures 4F, G). Finally, we developed the CuAGS-13- and ccA/ccB/ClearCode34-based nomograms to predict RFS in the TCGA cohort of ccRCC (I – IV) (Figures 4H, I). The comparison of these two nomograms showed that the CuAGS-13 score-based nomogram exhibited a much higher consistence between predicted and observed recurrences (Figures 4H, I, left panels).

The association between genomic alterations and the CuAGS-13 score in ccRCC

We next wanted to probe a potential link between the CuAGS-13 score and genomic alterations. Genomic data were available in 330 of 525 ccRCC tumors and 271 of them (81.12%) carried somatic mutations. The mutational landscape with 4% or more mutated genes was shown in Figure 5A. The following results were obtained from the analysis of ccRCC genomic alterations: (i) The CuAGS-13 score was significantly correlated with tumor mutation burden (TMB) in a positive manner (Figure 5B), and high-risk score tumors carried significantly a significantly higher frequency of BAP1 and SETD2 mutations (Figure 5C). (ii) The score and aneuploidy correlated positively with each other (Figure 5D). (iii) Homologous recombination deficiency (HRD) was highly correlated with the risk score (Figure 5E). In addition, intratumor heterogeneity (ITH), one of the key drivers for ccRCC evolution (38), was highly correlated with the CuAGS-13 score (Figure 5F).

The enriched signaling pathways in ccRCC tumors with high CuAGS-13 score

We further sought to determine differences in signaling pathways between CuAGS-13 high and low risk tumors. In the TCGA cohort, the GSEA-KEGG and Hallmark analyses revealed 31 and seven pathways enriched in CuAGS-high tumors, respectively (Figure S6), and these pathways were mainly involved in metabolisms. Unexpectedly, the TCA cycle and oxidative phosphorylation were also significantly enriched in this group of tumors. The analysis of the E-MTAB-1980 cohort showed very similar results (Figure S7).

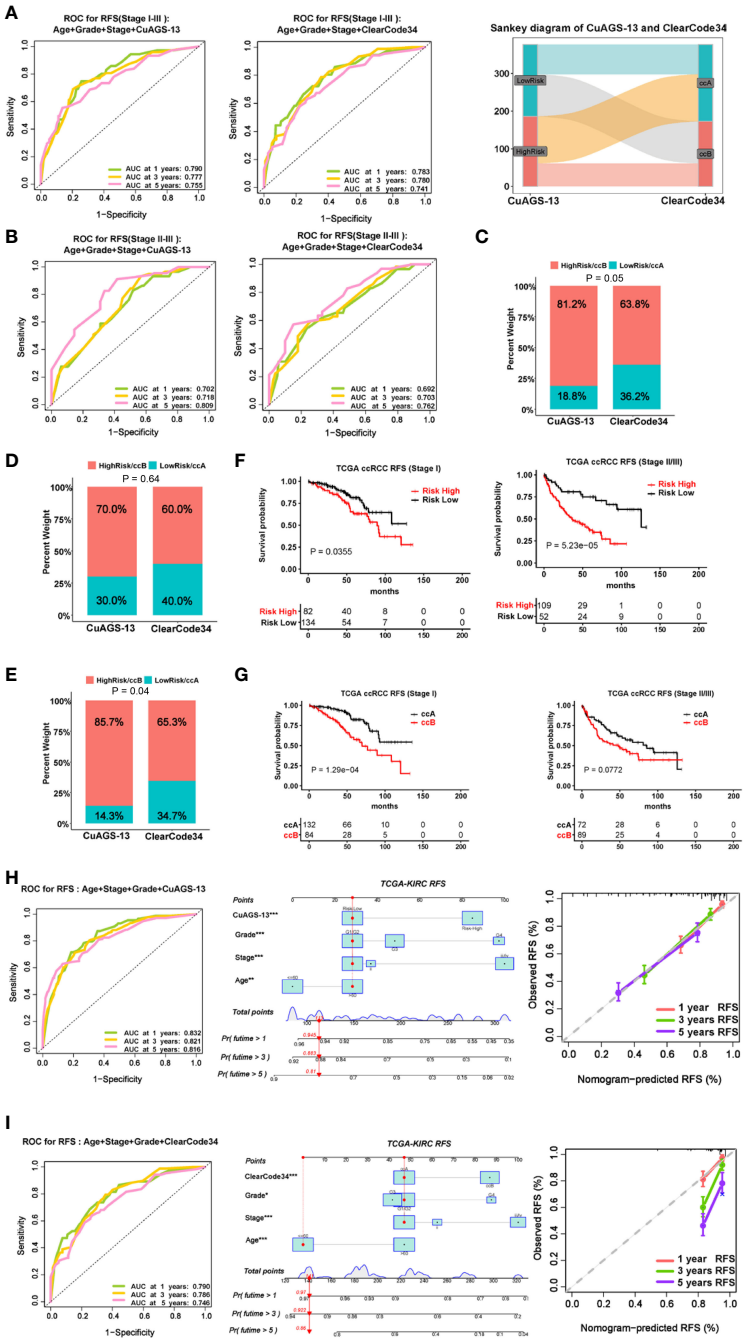

FIGURE 4
Comparison of predictive powers for recurrence and recurrence-free survival (RFS) between the CuAGS-13 and ccAccB/Clearcode34 models. **(A)** The ROC curve showing accuracy in predicting 1-, 3- and 5-year RFS for patients at stage I – III using CuAGS-13 (Left) and Clearcode34 (Middle) models. Right: The Sankey diagram showing different patient groups classified the CuAGS-13 and ccAccB/Clearcode34 models. **(B)** Left: The ROC curve showing accuracy in predicting 1-, 3- and 5-year RFS for patients at stage II – III using CuAGS-13 (Left) and ccAccB/Clearcode34 (Right) models. **(C)**: C-index analysis showing higher sensitivity of CuAGS-13 than Clearcode34 models for predicting recurrence in all patients at stage I – III. **(D)** C-index analysis showing no significant differences by CuAGS-13 and Clearcode34 models for predicting recurrence in patients at stage I **(E)** C-index analysis showing higher sensitivities of the CuAGS-13 than Clearcode34 models for predicting recurrence in stage II-III patients. **(F, G)** Kaplan–Meier survival analysis showing RFS predictive powers of CuAGS-13 **(F)** and Clearcode34 **(G)** models in patients at stage I and stage II – III, respectively. **(H)** The CuAGS-13 model-based nomogram for predicting 1-, 3- and 5-year RFS in TCGA ccRCC patients (stage I – IV). **(I)** The ccAccB/Clearcode34 model-based nomogram for predicting 1-, 3- and 5-year RFS in TCGA ccRCC patients (stage I – IV).

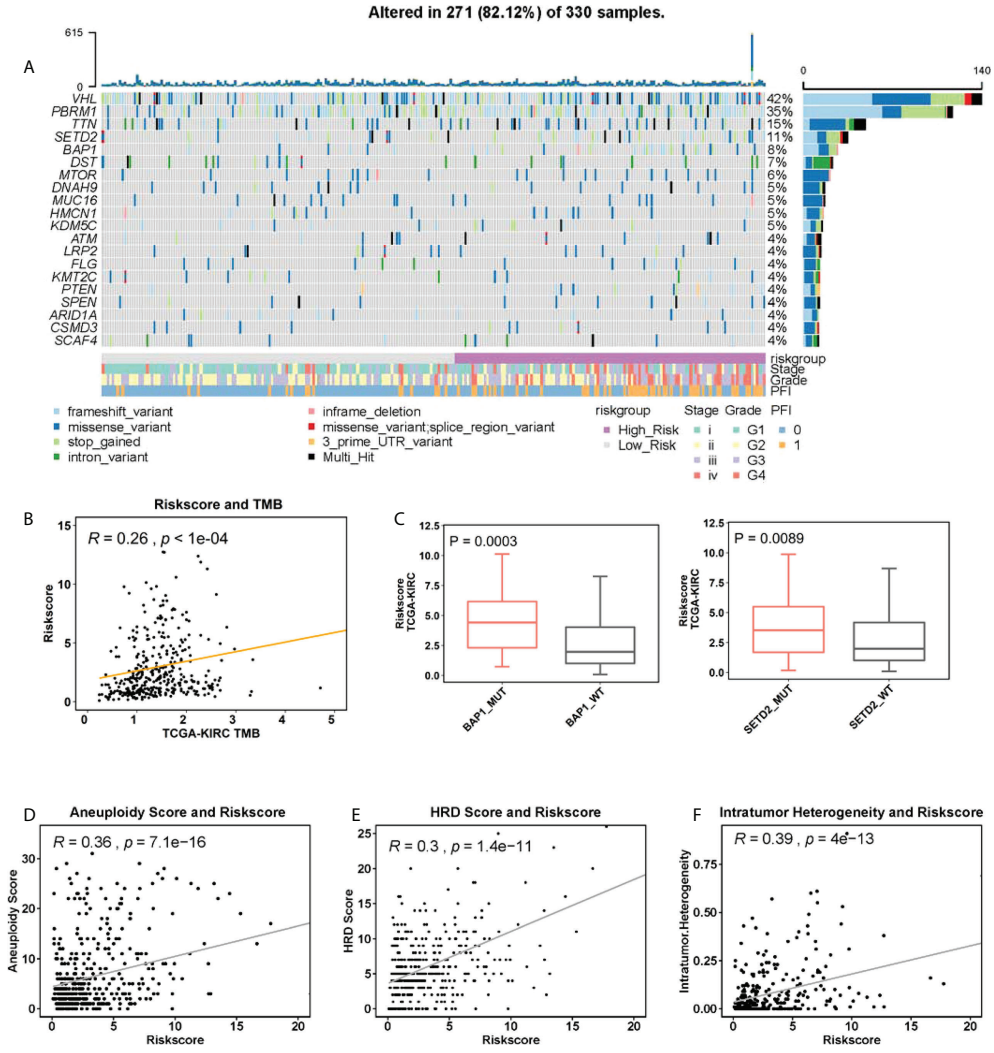

FIGURE 5
The association between genomic alterations and CuAGS-13 score in ccRCC. **(A)** The overview of the somatic mutations and relation to the CuAGS-13 score and clinical-pathological variables in the TCGA ccRCCs. **(B)** The positive correlation between CuAGS-13 score and tumor mutation burden (TMB) in the TCGA ccRCCs. **(C)** ccRCC tumors harboring BAP1 and SETD2 mutations exhibit significantly higher CuAGS-13 scores. **(D)** Positive correlation between the CuAGS-13 score and aneuploidy in ccRCC tumors. **(E)** Positive correlation between the CuAGS-13 score and homologous recombination deficiency (HRD) in ccRCC tumors. **(F)** Positive correlation between the CuAGS-13 score and intratumor heterogeneity in ccRCC tumors.

The CuAGS-13 score as a predictor for response to ICI therapy or combination with Bevacizumab

ICI therapy has been applied to ccRCC patients, but there is still lack of established biomarkers reliably predicting response. We sought to evaluate whether the CuAGS-13 risk score could serve as such a predictor. The IMmotion150 phase II trial (34, 35), which included 263 ccRCC patients, was analyzed as the training cohort (Table S4). Among these patients, 86 received Atezolizumab therapy, 88 were treated with Atezolizumab in combination with Bevacizumab, and the rest 89 with Sunitinib. We first analyzed 86 patients treated with atezolizumab alone. Patient responses to Atezolizumab were divided into complete/partial remission (CRPR), stable disease (SD) and progressive disease (PD). CRPR, SD and PD in the high-risk group were 14.6%, 39% and 46.3%, respectively, while 35%, 47.5% and 17.5% in the low-risk group, respectively (P = 0.011) (Figure 6A). The median PFS for high- and low-risk groups were 3 and 14.1 months, respectively (P = 0.004, HR, 2.6 (1.61 – 4.65)) (Figure 6A). For 88 patients treated with both Atezolizumab and Bevacizumab, the CRPR rate increased robustly from 14.6% to 30.2% in the high-risk group patients, while was largely same in the low-risk group (35% *vs.* 36.6%) (Figure 6B). Nevertheless,

patients with PD during the treatment were 3-time higher in the high- than low-risk groups (46.5% *vs.* 14.6%; P = 0.004) (Figure 6B); and the median PFS for high- and low-risk groups were 5.3 and 14.9 months, respectively (P = 0.025, HR, 1.8 (1.06 – 3.01)) (Figure 6B). Of note, in the CuAGS-13 high-risk group, the median PFS increased from 3.0 to 5.3 months when Bevacizumab was added, but this PFS increase was not statistically significant compared with that in patients treated with Atezolizumab alone (P = 0.20; HR, 1.36 (0.83 – 2.22)). Patients receiving Atezolizumab alone and plus Bevacizumab were then analyzed together, and increased CRPR while decreased SD rates were the major changes in the high-risk group compared with those in patients treated with Atezolizumab alone. The treatment results between high- and low-risk groups were CRPR, 22.6% and 35.8%; SD, 29.8% and 49.4%; PD, 47.6% and 14.8%, respectively (P = 6.7e-05) (Figure 6C). The median PFS for high- and low-risk group patients were 3.1 and 14.3 months, respectively (P = 1.6e-05; HR, 2.24 (1.52 – 3.29)) (Figure 6C). To probe how the CuAGS-13 score affects the efficacy of ICI therapy, we analyzed its relationship with Tumor Immune Dysfunction and Exclusion (TIDE) score, a computational framework to predict responses to immune checkpoint blockade and determine mechanisms underlying tumor immune escape (37). As shown in Figure 6D, the total TIDE score was significantly higher in the high-risk group. Consistently, exclusion, MDSC and CAF scores except M2 score were all higher in this group, whereas there were no differences in Dysfunction score between two groups (Figure 6D).

For validation, 120 ccRCC patients who received Nivolumab treatment in the CheckMate025 phase II trial (23, 24) were analyzed for their efficacy (Table S5). CRPR, SD and PD in the high-risk group were 13%, 37% and 50%, respectively, while 31.6%, 43.9% and 24.6% in the low-risk group, respectively (P = 0.01) (Figure 6E). The better efficacy in the low-risk group led to significantly longer patient OS and PFS (Figure 6E). The median PFS in the high- and low-risk groups was 2.1 and 7.2 months, respectively (P = 0.0003; HR, 1.98 (1.33 – 2.94) (Figure 6E), while OS was 17.9 and 38.4 months, respectively (P = 0.004; HR, 1.87 (1.21 – 2.89) (Figure 6E). These results were largely in accordance with those obtained from IMmotion150. There were no differences in the total TIDE score and T cell dysfunction score, however, T-cell exclusion, MDSC and CAF scores were significantly higher in the high-risk group (Figure 6F), which was consistent with the analysis result obtained from IMmotion150.

To further determine the relationship between the CuAGS-13 and TIDE scores, we analyzed the TCGA cohort of ccRCC. The total TIDE, dysfunction, exclusion, MDSC and CAF scores were all significantly higher, while TAM M2 score was lower in the high-risk group (Figure 6G). These findings favor an increased TIDE score in the CuAGS-13 high-risk group patients.

The CuAGS-13 score as a predictor for response to Sunitinib treatment

We also evaluated whether the CuAGS-13 score model could predict the efficacy in patients treated with Sunitinib. As documented above, Sunitinib was applied to 89 patients in the IMmotion150 cohort (34, 35), and the analysis results showed that the total CR and PR rate was more than 4-fold higher in the low- than high-risk groups (47.4% *vs.* 13.6%) (P = 0.004) (Figure 7A). The median PFS for high- and low-risk groups was 5.8 and 11 months, respectively (P = 0.03; HR, 1.77 (1.04 – 3.03) (Figure 7C). In the second cohort of 416 ccRCC patients treated with Sunitinib (IMmotion151) (18, 36) (Table S6), we obtained similar results (low- *vs.* high-risk: CRPR, 44.3% *vs.* 28.8%; SD, 43.8% *vs.* 42.4%; PD, 11.9 *vs.* 28.8%. P = 0.0004) (Figure 7B). In accordance with the findings above, patient PFS was significantly shorter in the high- than low-risk groups, and median PFS was 5.8 and 13.9 months, respectively (P = 5.0e-12; HR, 2.26 (1.78 – 2.88)) (Figure 7C). To determine whether Sunitinib affects the cuproptosis signaling, we analyzed the cuproptosis score in tumors derived from patient-derived xenografts (PDX) models in GSE64052 (39). Microarray data were available in five untreated and four Sunitinib-resistant PDX tumors and expression levels of 10 cuproptosis genes were listed in Table S7. As shown in Figure 7D, the Sunitinib-resistant tumors expressed lower cuproptosis scores than untreated ones, however, the difference was not statistically significant.

Discussion

Approximately 30% of ccRCC patients with localized disease relapses after nephrectomy, and therefore stratifying recurrence risk is important, especially for patients at stage II and III whose clinical behaviors are precarious (2, 4). On the other hand, up to 30% ccRCC patients present metastasis at diagnosis and systemic treatments are required (2, 4). During the last decade, tyrosine-kinase inhibitors such as Sunitinib, VEGF antibody Bevacizumab and ICIs have been applied to metastatic or relapsed patients and good efficacy observed in a subset of ccRCCs (15, 17). Reliable biomarkers are required to accurately stratify recurrence risk, and to predict response to targeted therapeutic drugs and ICIs. In the present study, we addressed these issues by analyzing ccRCCs from the TCGA and other datasets to construct a cuproptosis-associated model for prediction of survival, recurrence and response to ICIs, Bevacizumab and Sunitinib.

Cuproptosis is copper-dependent cell death resulting from FDX1-mediated mitochondrial protein lipoylation. FDX1 reduces Cu^{++} to Cu^{+}, while lipoic acid pathway effectors, especially lipoyl synthase (LIAS), together with FDX1, promote the lipoylation of the pyruvate dehydrogenase (PDH) complex-containing enzymes in the TCA cycle (32). The PDH

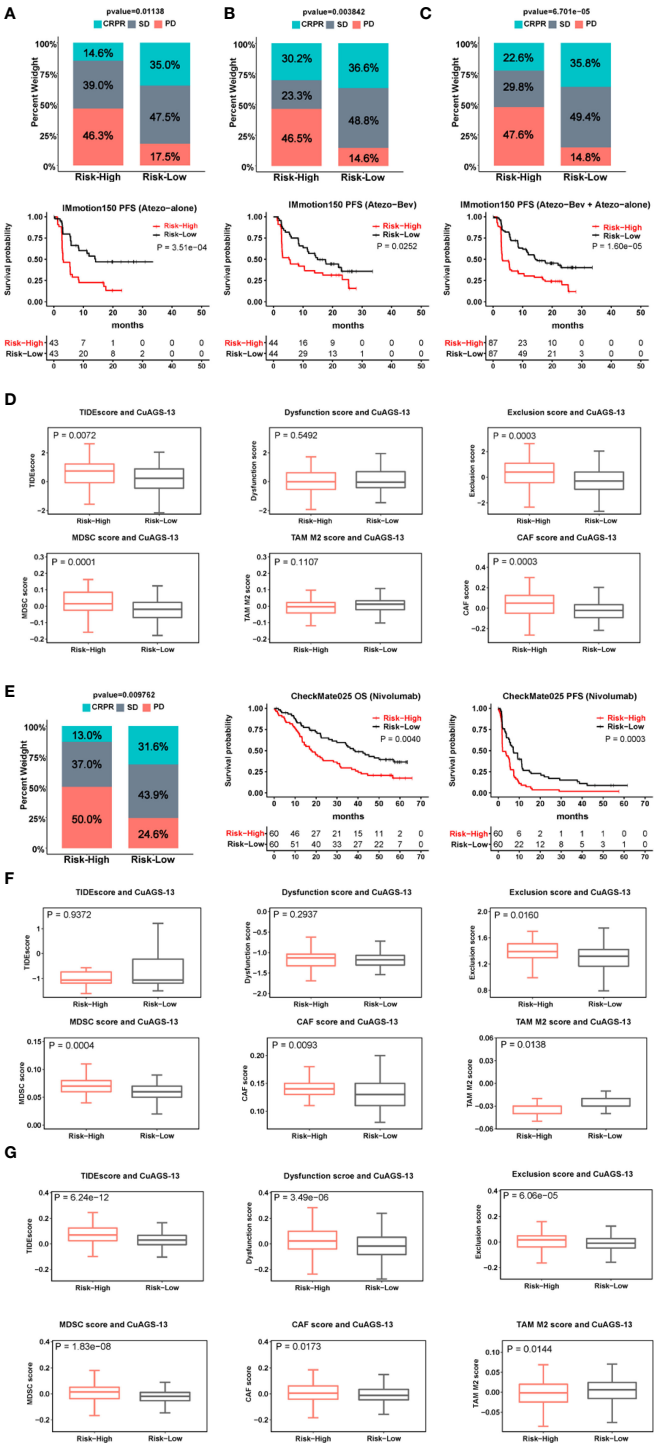

FIGURE 6
The CuAGS-13 score prediction of patient response to immune checkpoint inhibitors (ICIs) and combination with Bevacizumab in ccRCC. **(A–C)** The CuAGS-13 score prediction of patient response to Atezolizumab alone or Atezolizumab plus Bevacizumab in IMmotion150 trial. Differences in response rates and PFS between the CuAGS-13 high- and low-risk group patients treated with Atezolizumab alone **(A)**, Atezolizumab plus Bevacizumab **(B)** and all together **(C)**. **(D)** TIDE score analyses showing differences between the CuAGS-13 high- and low-risk group patients in IMmotion150 trial. **(E)** Differences in response rates and survival (OS and PFS) between the CuAGS-13 high- and low-risk group patients treated with Nivolumab in CheMate025 trial. **(F)** TIDE score analyses showing differences between the CuAGS-13 high- and low-risk group patients in CheMate025 trial. **(G)** TIDE score analyses showing differences between the CuAGS-13 high- and low-risk group patients in the TCGA ccRCC cohort.

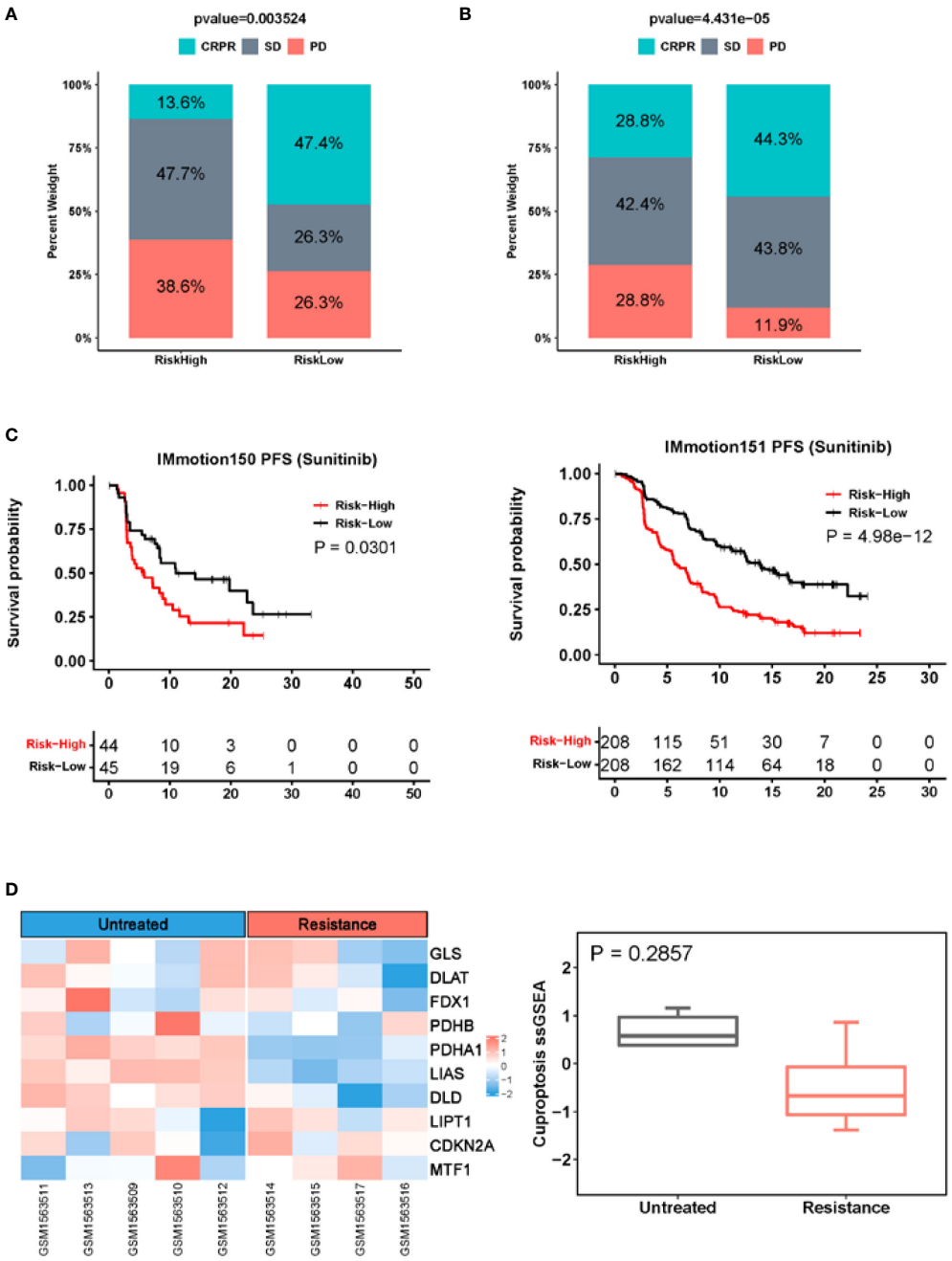

FIGURE 7
The CuAGS-13 score prediction of patient response to Sunitinib in ccRCC. **(A)** Differences in response rates between the CuAGS-13 high- and low-risk group patients treated with Sunitinib in IMmotion150 trial. **(B)** Differences in response rates between the CuAGS-13 high- and low-risk group patients treated with Sunitinib in IMmotion151 trial. **(C)** Significant association between shorter PFS and the CuAGS-13 high-risk group patients treated with Sunitinib in IMmotion150 trial (left) and IMmotion151 trial (right). **(D)** The lower cuproptosis score in Sunitinib-resistant PDX tumors. Microarray data in five untreated and four Sunitinib-resistant PDX tumors were analyzed for their cuproptosis score. Left panel: Heatmap showing expression of 10 cuproptosis factors. Right panel: The cuproptosis score in untreated and Sunitinib-resistant PDX tumors. A cuproptosis score was calculated using ssGSEA.

complex includes dihydrolipoamide S-acetyltransferase (DLAT), pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1), and pyruvate dehydrogenase E1 subunit beta (PDHB), and their lipoylation is required for enzymatic function (32). However, Cu^+ directly binds to the lipoyl moiety in these lipoylated proteins, and if excessively accumulated, results in lipoylated protein aggregation, proteotoxic stress and eventual cell death (32). In addition, FDX1 and Cu^+ facilitates degradation of Fe–S cluster proteins, which further enhances onsets of cuproptosis (32). It is currently unclear whether cuproptosis, like apoptosis or other types of RCD, has any roles in oncogenesis. Our analyses of the TCGA cohort of ccRCC showed that higher FDX1 expression is significantly associated with longer OS and PFS, and moreover, its downregulation occurs in ccRCC, which collectively indicates that cuproptosis may act as tumor suppressor in this cancer type. Moreover, according to correlation with cuproptosis factors, we identified a panel of cuproptosis-associated genes and developed the CuAGS-13 score model that could predict patient OS/PFS and recurrence risk with a high accuracy.

Gene expression patterns have been shown to improve cancer classification and prediction of patient outcomes, and several groups have developed expression profiling-based molecular tools for ccRCC prognostication (5–10, 40). For instance, Rini et al. introduced a 16-gene score for recurrence risk stratification in ccRCC patients at stage I - III (10), and Buttner et al. set up the S-3 score (the 97 gene signature based on gene expression in the terminal part of proximal tubules) for survival assessment (8). More recently, a 13-gene signature was constructed to predict risk and survival of ccRCCs. However, these expression signatures have not been well validated independently. There is another molecular classification score so-called ccA/ccB/ClearCode34 model (6, 7), which have been evaluated in several clinical observations, and consistently showed their robustness in outcome prediction of ccRCC (41). To test the stratification ability of the CuAGS-13 score, we further analyzed the same cohorts of ccRCCs from TCGA and E-MTAB-1980 using the ClearCode34 score and compared the predictive effectiveness as assessed by both models. The obtained results demonstrated that the CuAGS-13 score outperformed the ClearCode34 classifier in predicting recurrence risk and RFS. Further studies of additional cohorts of ccRCC are required to confirm the present findings.

Efforts have been made to search for predictors of ICI response in ccRCC patients, and several molecules are shown to be useful in some reports but fail to be validated by others (22–27, 42, 43). Intriguingly, the presence of high CD8 T cells in tumors is associated with poor prognosis (16). More recently, other biomarkers have been introduced to predict response to ICIs (28). Here we observed that the CuAGS-13 score assessment helped stratify ICI responders in two cohort patients who received either Atezolizumab or Nivolumab. In both cohorts, the CRPR rate was more than two-fold higher in the low- than high-risk group. Consistently, patients in the low-risk group had significantly longer PFS. The TIDE analysis revealed significantly higher T cell exclusion, MDSC and CAF scores while lower TAM M2 score in the high-risk group from both cohorts, which may contribute to poor response to ICIs in this group. It is currently unclear whether cancer immunotherapy is involved in cuproptosis. Recent studies showed that CD8 T cells promoted tumor cell lipid peroxidation and ferroptosis in patients treated with nivolumab (44, 45). It is thus worth probing the mechanistic relationship between cuproptosis and ICI efficacy, and if this is indeed the anti-tumor mechanism underlying ICI immunotherapy, targeting the cuproptosis pathway in combination with ICIs may be a novel therapeutic strategy.

Combination of anti-angiogenic therapy and ICIs has been shown to synergistically inhibit tumor growth and progression (19, 20). Targeting angiogenesis convert the tumor-immune environment from immune-suppressive to immune-supportive, thereby promoting the efficacy of ICIs. On the other hand, ICIs exert an anti-angiogenic effect (19, 20). However, it remains poorly defined which patients will benefit from this combination protocol (19). Interestingly, we observed that the combined treatment of Atezolizumab and Bevacizumab doubled a CRPR rate in CuAGS-13 high-risk group patients without improving the efficacy in low-risk group patients. Moreover, the increased CRPR seen in the high-risk group with the combined therapy was mainly derived from SD patients, because the PD rate was largely same between patients treated with Atezolizumab alone and Atezolizumab plus Bevacizumab. Based on the present findings, the Atezolizumab/Bevacizumab combination is suggested to apply to the CuAGS-13 high-risk patients.

Sunitinib has been widely used for ccRCC treatment (18, 36). We observed that patients who acquired CRPR were more than 3-time higher in the low-risk group compared with those in the high-risk group in IMmotion150 cohort treated with Sunitinib. The analysis of IMmotion151 cohort similarly showed significantly higher numbers of CRPR patients coupled with longer PFS in the low-risk group. These findings strongly suggest that the CuAGS-13 model can be used to predict Sunitinib responders in ccRCCs. It is currently unclear whether Sunitinib is associated with cuproptosis induction. Our preliminary results of GSE64052 PDX tumor analyses showed that Sunitinib-resistant tumors tended to have a diminished cuproptosis score, indicating possible escape of cuproptosis. Further cellular experiments and comparison of cuproptosis between Sunitinib-sensitive and resistant tumors are required to draw solid conclusions.

In our investigations, ccRCC tumors carrying BAP1 and SETD2 mutations exhibited higher CuAGS-13 scores. BAP1 is responsible for deubiquitinating H2K119, thereby impairing regulatory function of the polycomb repression complex1 (PRC1) in transcription, while SETD2 demethylate H3K36,

leading to altered gene transcription (11, 38). During the ccRCC evolution, both BAP1 and SETD2 act as drivers for disease progression (38). It will be interesting to explore whether their mutations result in dysregulation of cuproptosis factors and escape of cuproptosis in ccRCCs. In addition, aneuploidy and HRD were significantly correlated with CuAGS-13 scores. Because BAP1 and SETD2 are required for genomic stability (38), their mutations may contribute to the correlation between them observed above. In addition, CuAGS-13 high-risk scores are significantly associated with male sex, senior age, higher grade tumors and advanced stages. Taken together, the CuAGS-13 model is a molecular classifier with many integrate features of ccRCC.

Metabolic reprogramming is a key feature of ccRCC due to the VHL inactivation and aberrant accumulation of HIF1/2α. Indeed, the GSEA analysis revealed that the enriched pathways were mainly involved in metabolic alterations in ccRCC tumors with CuAGS-13 high-scores, however, the enrichment of TCA and oxidative phosphorylation pathways were also observed in these tumors, which was unexpected. It was observed that SETD2 loss triggered a switch from glycolysis to OXPHS in ccRCC cells (46), while tumors with high CuAGS-13 score exhibited higher frequencies of SETD2 mutations, which might provide potential explanation. Likely, other unknown factors make contributions, too and further studies are required to elucidate this issue.

In summary, cuproptosis is the newly identified form of RCD, and based on its signaling molecules, we developed the CuAGS-13 score model that provides a robust tool to predict patient survival, recurrence, and response to ICIs, Bevacizumab and Sunitinib in ccRCC. This model, although derived from cuproptosis-related genes, is a classifier integrated with molecular and many other features of ccRCC. The present findings strongly suggest that the CuAGS-13 score system might significantly improve patient stratification for precision medicine of ccRCC, and it is worthy of validating these observations in clinical practices.

Data availability statement

Source data downloaded from public databases are provided with this paper. Data from the IMmotion150 and151 trial were downloaded from European Genome-Phenome Archive (EGA) under accession number EGAS00001002928 and EGAC00001001813 with EGA approval. Any additional information required to reanalyze the data reported in this paper is available from the corresponding authors upon reasonable request.

Author contributions

HY, YF, and DX conceived and designed the study. HY, JW, XQ, and QY performed bioinformatics analysis. YF and DX supervised the study. HY, YF, and DX wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.971142/full#supplementary-material

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The prognostic value of baseline and early variations of peripheral blood inflammatory ratios and their cellular components in patients with metastatic renal cell carcinoma treated with nivolumab: The Δ-Meet-URO analysis

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Background: Treatment choice for metastatic renal cell carcinoma (mRCC) patients is still based on baseline clinical and laboratory factors.

Methods: By a pre-specified analysis of the Meet-URO 15 multicentric retrospective study enrolling 571 pretreated mRCC patients receiving nivolumab, baseline and early dynamic variations (Δ) of neutrophil, lymphocyte, and platelet absolute cell counts (ACC) and their inflammatory ratios (IR) were evaluated alongside their association with the best disease response and overall (OS) and progression-free survival (PFS). Multivariable analyses on OS and PFS between baseline and Δ ACC and IR values were investigated with receiving operating curves-based cut-offs.

Results: The analysis included 422 mRCC patients. Neutrophil-to-lymphocyte ratio (NLR) increased over time due to consistent neutrophil increase ($p < 0.001$). Higher baseline platelets ($p = 0.044$) and lower lymphocytes ($p = 0.018$), increasing neutrophil Δ (p for time-group interaction <0.001), higher baseline IR values (NLR: $p = 0.012$, SII: $p = 0.003$, PLR: $p = 0.003$), increasing NLR and systemic immune-inflammatory index (SII) (i.e., NLR x platelets) Δ (p for interaction time-group = 0.0053 and 0.0435, respectively) were associated with disease progression. OS and PFS were significantly shorter in patients with baseline lower lymphocytes ($p < 0.001$ for both) and higher platelets ($p = 0.004$ and $p < 0.001$, respectively) alongside early neutrophils Δ ($p = 0.046$ and $p = 0.033$, respectively). Early neutrophils and NLR Δ were independent prognostic factors for both OS ($p = 0.014$ and $p = 0.011$, respectively) and PFS ($p = 0.023$ and $p = 0.001$, respectively), alongside baseline NLR ($p < 0.001$ for both) and other known prognostic variables.

Conclusions: Early neutrophils and NLR Δ may represent new dynamic prognostic factors with clinical utility for on-treatment decisions.

KEYWORDS

renal cell carcinoma, immunotherapy, dynamics, inflammatory, NLR, prognostic

1 Introduction

Immune checkpoint inhibitors (ICIs) have reshaped the treatment landscape of metastatic renal cell carcinoma (mRCC) with the introduction of nivolumab in pretreated patients in 2015 and the more recent first-line immunotherapy-based combinations (1–3).

Despite the survival benefit leading to these new immunotherapy indications, the proportion of mRCC patients achieving long-term benefits from ICI-based therapies is still low. Early predictive biomarkers are needed to optimize patient and treatment selection (4, 5). The programmed-cell-death-ligand1 (PD-L1) expression, tumor mutational burden (TMB), and tumor microenvironment-related signatures have been investigated for their prognostic and predictive value. However, none has still reached sufficient evidence or applicability to be routinely tested in everyday clinical practice (6–9). Although PD-L1 expression correlated with poor prognosis and advanced clinicopathological features in RCC patients (10–12), it is expressed in about one quarter of patients with clear-cell RCC and approximately 10% of those with non-clear cell RCC (10) and does not seem to have a predictive value (13).

Inflammatory ratios (IR) from peripheral blood might reflect the cancer-related inflammatory phenomena, the host immune response to cancer and comorbidity (14). In practically every area of medicine, including cancer patients, elements of the full blood count, like the total leukocyte, neutrophil, lymphocyte, monocyte, and platelet counts, have been extensively studied as a proxy of a dysfunctional pro-inflammatory response (15–17). It has long been known that blood count parameters have a prognostic value for mRCC. High neutrophils were initially reported as a poor prognostic indicator in 1996 (18). Later, the notion of neutrophils-to-lymphocytes ratio (NLR) reached the clinical practice (19). No later than 2011, the relevance of an elevated platelet count was recognized (20). IR have emerged as a quick and inexpensive assessment with reproducible prognostic value across different tumor types, stages, and treatment settings, particularly for patients with metastatic tumors treated with ICIs (21, 22). However, their baseline value has been mainly investigated so far, while increasing evidence suggests a possible correlation with disease outcome related to their early variations during treatment, particularly in lung cancer patients (23–33). If associated with worse prognosis and failure of therapy their early variations might have clinically helpful aftermaths, like the anticipation of disease reassessments during treatments and an earlier start of the next treatment line. Furthermore, a better understanding of the IR specific cellular component on-treatment variations would shed light on the shift of the patient's immune system in response to anti-tumoral treatments, specifically the ICIs.

The Meet-URO 15 study is one of the largest analyses of baseline prognostic factors, including IR in patients with mRCC treated with ICIs (34). This study developed a novel prognostic score, namely the Meet-URO score, based on the addition of two newly identified independent variables, or the NLR and the presence of bone metastases.

In this pre-specified sub-analysis of the Meet-URO 15 study, we longitudinally investigated the dynamics of neutrophil, lymphocyte and platelet absolute cell counts (ACC) and IR during the first four nivolumab treatment administrations and their correlation with response and survival.

2 Materials and methods

The analysis was a pre-specified secondary analysis of the multicentric retrospective Meet-URO 15 study, approved by the institutional review board (regional ethical committee of Liguria – registration number 068/2019). The Meet-URO 15 study was conducted among 34 Italian centers and enrolled 571 mRCC patients. It was performed according to the Declaration of Helsinki. All living patients signed written informed consent.

2.1 Study population and treatment

Patients with mRCC who had received at least two completed nivolumab administrations as ≥2nd treatment line between October 2015 and November 2019 were included in the analysis. Nivolumab was administered intravenously at the dose of 3 mg/kg every 2 weeks until May 2018, then at the fixed dose of 240 mg every 2 weeks, or 480 mg every 4 weeks, according to the clinical practice of each participating center. The treatment was continued until progressive disease (PD), unacceptable toxicity, death, or patient choice. Patients with radiological PD were allowed to continue therapy beyond progression of clinical benefit according to physicians' decision.

The follow-up consisted of periodic physical examinations, laboratory analyses, and imaging assessments. Radiological assessments included computed tomography (CT) scan of chest-abdomen-pelvis and head (when clinically indicated) at baseline and every 2–4 months thereafter, according to physicians' practice, or when PD was clinically suspected.

2.2 Absolute cell counts and inflammatory ratios from peripheral blood

Data from full blood counts performed within 7 days from each of the first four nivolumab administrations were collected, including neutrophils, lymphocytes and platelets ACC, and the following IR: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and the systemic immune-inflammation

index (SII, calculated as NLR × platelets as originally developed) (35). Patients were then followed up until the date of the database lock for the final analysis on 31 July 2020.

2.3 Study objectives and endpoints

The first study objective was the description of the ACC and IR value variations through the first four nivolumab administrations (Delta, Δ). The Delta was derived from subtracting the parameter value at the fourth nivolumab administration minus baseline level. The second study objective evaluated the correlation between ACC and IR baseline and Δ values with the best disease response to treatment. The third study objective included the correlation of their baseline and early Δ values with overall survival (OS) and progression-free survival (PFS), the assessment of related prognostic models and potential interactions between baseline and Δ values on OS and PFS. Early Δ was defined as the variations of values from the first to the second treatment administrations, or the subtraction of the parameter value at the second nivolumab administration minus baseline level. The disease response to treatment was defined in each center, referring to the Response Evaluation Criteria in Solid Tumours (RECIST) criteria version 1.1 as complete response (CR), partial response (PR), stable disease (SD) and PD (36). Responders were defined as those patients achieving CR or PR as the best disease response. OS was calculated from the first nivolumab administration until death, censored at last follow-up for living patients, while PFS was calculated from the first nivolumab administration until PD or death, censored at last follow-up for patients who did not progress and were alive at the end of the follow-up.

2.4 Statistical analysis

Patients' characteristics were reported using absolute frequency and percentage for categorical variables and by mean with standard deviations, or median and ranges, for quantitative variables.

Analysis of variance (ANOVA) was used to test differences between baseline ACC, IR, and the best response to treatment; p values for each comparison (i.e., PD vs. CR/PR) were adjusted using the false discovery rate approach for multiple comparisons.

The longitudinal trend of ACC and IR was assessed using the linear mixed model with random intercept; p-values for longitudinal trends were corrected for multiple comparisons using the false discovery rate approach. The interaction between the therapy administration number and best response to treatment was performed to test differences across administrations between CR/PR, SD and PD, and ACC or IR.

The Kaplan–Meier method was used to estimate survival curves of OS and PFS by the baseline and early ACC and IR Δ values.

Survival receiver operating curves (ROC) based on OS were performed to identify both baseline ACC and early ACC and IR Δ cut-off values; baseline IR cut-offs were those identified in the previous analysis (34).

Univariable and multivariable analyses to test the association between baseline, early ACC, and IR Δ values and PFS and OS were performed using the Cox proportional hazard regression model. As the early Δ was the variable of interest, multivariable models were performed only for those values with a p value <0.10 at the univariable analyses. All the other characteristics, including the International Metastatic RCC Database Consortium (IMDC) risk score for mRCC and the presence of bone metastases, were also considered into the model when a p value <0.10 was found at the univariable analysis.

The interaction between baseline and early Δ values was assessed to test whether the association with outcomes depended on baseline values. The level of significance was set to 0.05.

All statistical analyses were performed using Stata v.16 (StataCorp 2019).

3 Results

3.1 Patients' characteristics

Four hundred twenty-two mRCC patients had available data for the analysis. The CONSORT flow diagram is shown in Figure 1S. Forty-two out of the 571 overall patients (7.4%) did not reach the second treatment cycle. Of 107 patients (18.7%) who received at least two treatment cycles, 40 and 67 had laboratory missing data at baseline or the second cycle, respectively, thus leading to the 422 patients included in the analysis. Their characteristics are reported in Table 1. Of the 422 patients, 309 (73.2%), 82 (19.4%), and 31 (7.4%) received nivolumab as a second-, third-, or further line treatment. Most patients had clear-cell histology (85%) and received nivolumab as a second line treatment (73%); median age was 63.4 years (range: 18–85). According to the prognosis estimation at metastatic disease onset, 34% of patients were at favorable, 60% intermediate, and 6.5% poor-risk by the IMDC classification, while 22% belonged to the Meet-URO score risk group 1, 43% to group 2, 23% to group 3, and 11% to group 4.

3.2 Absolute cell count and inflammatory ratio variations during treatment

The ACC and IR Δ values through the first four nivolumab administrations are represented in Figure 1. Among the formers, the neutrophil counts consistently increased from baseline (mean: 4313 x10e3/L) to the fourth administration (mean: 5058x10e3/L) with a significant positive Δ at each therapy administration ($p < 0.001$; Figure 1A).

TABLE 1 Patients' characteristics.

Patients n = 422	
Characteristics	***N (%)***
Gender	
Male	305 (72.3)
Female	117 (27.7)
Median age, years (range)	63.4 (18-85)
<70	314 (74.4)
≥70	108 (25.6)
Karnofsky performance status	
≥80%	367 (87.0)
<80%	55 (13.0)
Histologic subtype	
Clear cell	358 (84.8)
Non-clear cell	64 (15.2)
Nephrectomy	
Yes No	376 (89.1)46 (10.9)
Metastatic ad diagnosis	
Yes	174 (41.2)
No	248 (58.8)
IMDC score at metastatic diagnosis	
Favorable	130 (33.9)
Intermediate	229 (59.6)
Poor	25 (6.5)
Missing	38
Meet-URO score	
1 (0-1)	92 (21.9)
2 (2-3)	182 (43.3)
3 (4-5)	98 (23.4)
4 (6-8)	48 (11.4)
5 (9)	0
Nivolumab line	
2nd line	309 (73.2)
3rd line	82 (19.4)
≥ 4th line	31 (7.4)
IMDC score at start of nivolumab	
Favorable	92 (21.9)
Intermediate	280 (66.7)
Poor	48 (11.4)
Missing	2
Lymph-nodal metastases	
Yes	226 (53.6)
No	196 (46.5)
Visceral metastases	
Yes	385 (91.2)
No	37 (8.8)
Bone metastases	
Yes	147 (34.8)
No	275 (65.2)

N, number of patients; IMDC, International Metastatic RCC Database Consortium.

After a non-significant initial drop below the baseline value (mean: 1492x10e3/L), lymphocyte counts progressively increased with a significant positive Δ reached at the fourth administration (mean: 1559x10e3/L) ($p = 0.030$) (Figure 1B).

A significant platelet positive Δ from baseline count (mean: 264x10e9/L) was observed at the second administration (mean: 292x10e9/L) ($p = 0.003$), followed by a non-significant drop with counts remaining higher than baseline until the fourth administration (mean: 276x10e9/L) (Figure 1C).

Reflecting trends of their constituting cell types, a significantly positive Δ was observed at each therapy administration time point for the NLR (from baseline mean 3.58 to 3.99 at the fourth; $p < 0.001$, $p = 0.037$, $p = 0.015$ at the second, third, and fourth, respectively) (Figure 1D), at the second only for SII (from mean 992 to 1260; $p < 0.001$) (Figure 1E) and PLR (from mean 209 to 244 at the second; $p = 0.001$) (Figure 1F).

3.3 Absolute cell counts and inflammatory ratios according to disease response

3.3.1 Baseline values

The baseline ACC and IR values according to the disease response to nivolumab are reported in Figure 2. Patients with PD had higher platelet (mean: 283x10e9/L) and lower lymphocyte (mean: 1401x10e3/L) baseline counts than responders (mean: 255 x10e9/L and 1610x10e3/L; $p = 0.044$ and $p = 0.018$, respectively) and higher baseline neutrophils (mean: 4707x10e3/L) and platelets (mean: 283x10e9/L) compared to patients with SD (mean: 3963x10e3/L and 250x10e9/L; $p = 0.003$ and $p = 0.036$, respectively) (Figures 2A–C).

Higher baseline NLR (mean: 4.12), SII (mean: 1208), and PLR (mean: 237) values were consistently associated with PD than responders (mean: 3.18, 836 and 184; $p = 0.012$, $p = 0.003$ and $p = 0.003$, respectively) or SD (mean: 3.35, 899 and 201; $p = 0.029$, $p = 0.014$ and $p = 0.032$, respectively) (Figures 2D–F).

3.3.2 Longitudinal variations (Δ)

The ACC and IR values Δ according to the disease response to therapy are represented in Figure 3.

Neutrophils significantly increased in patients with PD (from baseline mean count of 4612x10e3/L to 6176x10e3/L at the fourth administration) compared to responders (from 4364x10e3/L to 4547x10e3/L) or patients with SD (from 3890x10e3/L to 4498x10e3/L) (p for time-group interaction < 0.001) (Figure 3A).

No significant differences in lymphocyte and platelet Δ were observed according to disease response ($p = 0.41$ and $p = 0.60$, respectively). However, the higher baseline counts of lymphocytes were maintained over treatment in responders

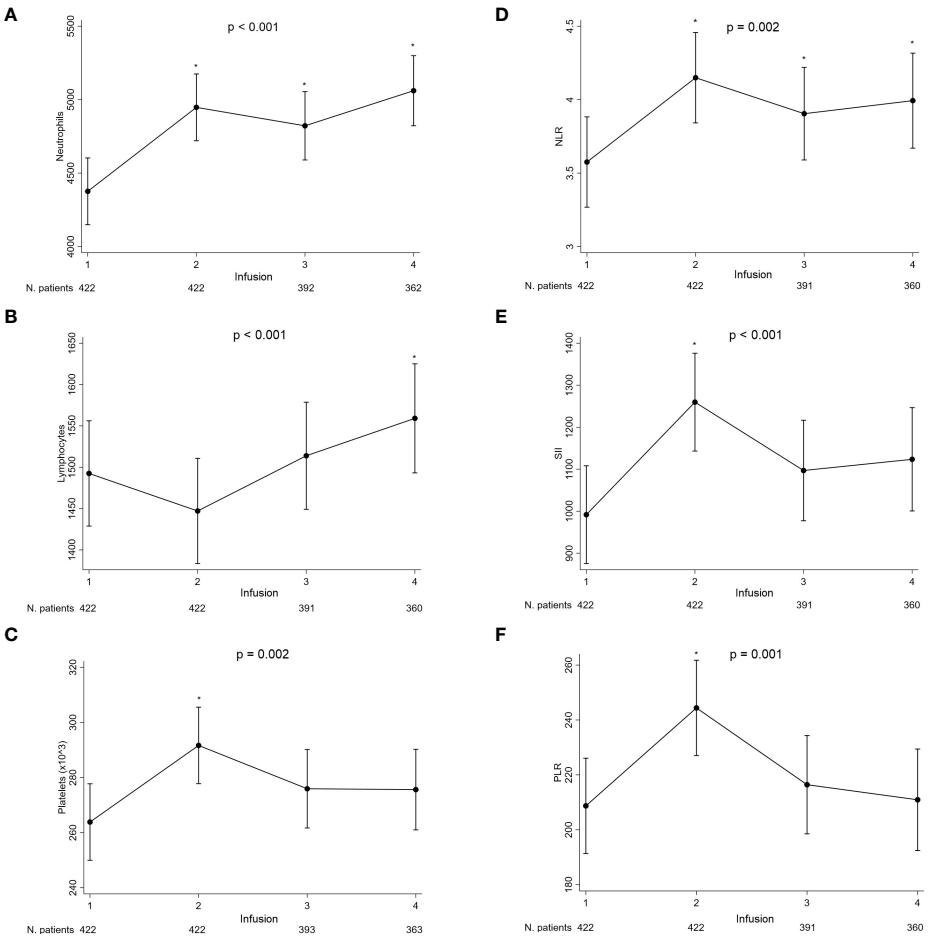

FIGURE 1
The ACC and IR Δ values through the first four nivolumab administrations.Neutrophils **(A)**, lymphocytes **(B)**, platelets **(C)**, NLR **(D)**, SII **(E)** and PLR **(F)** were assessed.*Significant difference compared with baseline and adjusted for multiple comparisons using the false discovery rate approach.

(from baseline mean count of 1591x10e3/L to 1653x10e3/L at the fourth administration) compared to patients with PD (from 1435x10e3/L to 1502x10e3/L) or SD (from 1476x10e3/L to 1525x10e3/L) (Figure 3B). Similarly, the higher baseline platelet counts were maintained in patients with PD (from 277x10e9/L to 298x10e9/L at the fourth administration) than responders (from 251x10e9/L to 255x10e9/L) or patients with SD (from 246x10e9/L to 266x10e9/L) (Figure 3C).

Accordingly, NLR and SII values significantly increased in patients with PD (from baseline mean value of 4.24 to 5.41 at the fourth administration for NLR, and from 1208 to 1618 for the SII) compared to responders (from 3.32 to 3.24 for NLR, and from 845 to 830 for SII) or patients with SD (from 3.35 to 3.55 for NLR, and from 883 to 973 for SII) (p for interaction time-group = 0.0053 and 0.0435 for NLR and SII, respectively) (Figures 3D, E). The PLR value Δ was not significantly increased according to the disease response (p for interaction time-group = 0.092) (Figure 3F).

3.4 Correlation of absolute cell counts and inflammatory ratios with survival outcomes

The univariable analyses of baseline and early ACC and IR Δ values, based on their ROC-based cut-off values, are reported in Table 2 and represented in Figure 4, 2S and 3S.

3.4.1 Baseline values

Higher baseline platelet (cut-off: ≥263x10e9/L) and lower lymphocyte (cut-off: <1460x10e3/L) counts were either

significantly associated with worse OS ($p < 0.001$ for both) and PFS ($p = 0.004$ and $p < 0.001$, respectively), while higher neutrophils (≥4330x10e3/L) were significantly associated with OS ($p < 0.001$) only and not with PFS ($p = 0.059$) (Table 2, Figure 1S and 2S).

Higher NLR (cut-off: ≥3.2), SII (cut-off: ≥720), and PLR (cut-off: ≥176) baseline values were associated with both worse OS ($p < 0.001$ for all) and PFS ($p < 0.001$ for all) (Table 2).

3.4.2 Longitudinal variations (Δ)

Increased neutrophil early Δ (cut-off: ≥730x10e3/L) only was either associated with OS ($p = 0.046$) or PFS ($p = 0.033$), while increased NLR early Δ (cut-off: ≥0.5) was significantly associated with PFS ($p = 0.007$) but not with OS ($p = 0.062$) (Table 2, Figure 4, Figure 5S and Figure 6S).

3.4.3 Multivariable analysis on survival outcomes

In two prognostic models by the NLR or neutrophil counts, higher baseline NLR values (cut-off: ≥ 3.2) ($p < 0.001$) or neutrophils (cut-off: ≥4330x10e3/L) ($p < 0.001$), increased early D of NLR (cut-off: ≥0.5) ($p = 0.014$) or neutrophils (cut-

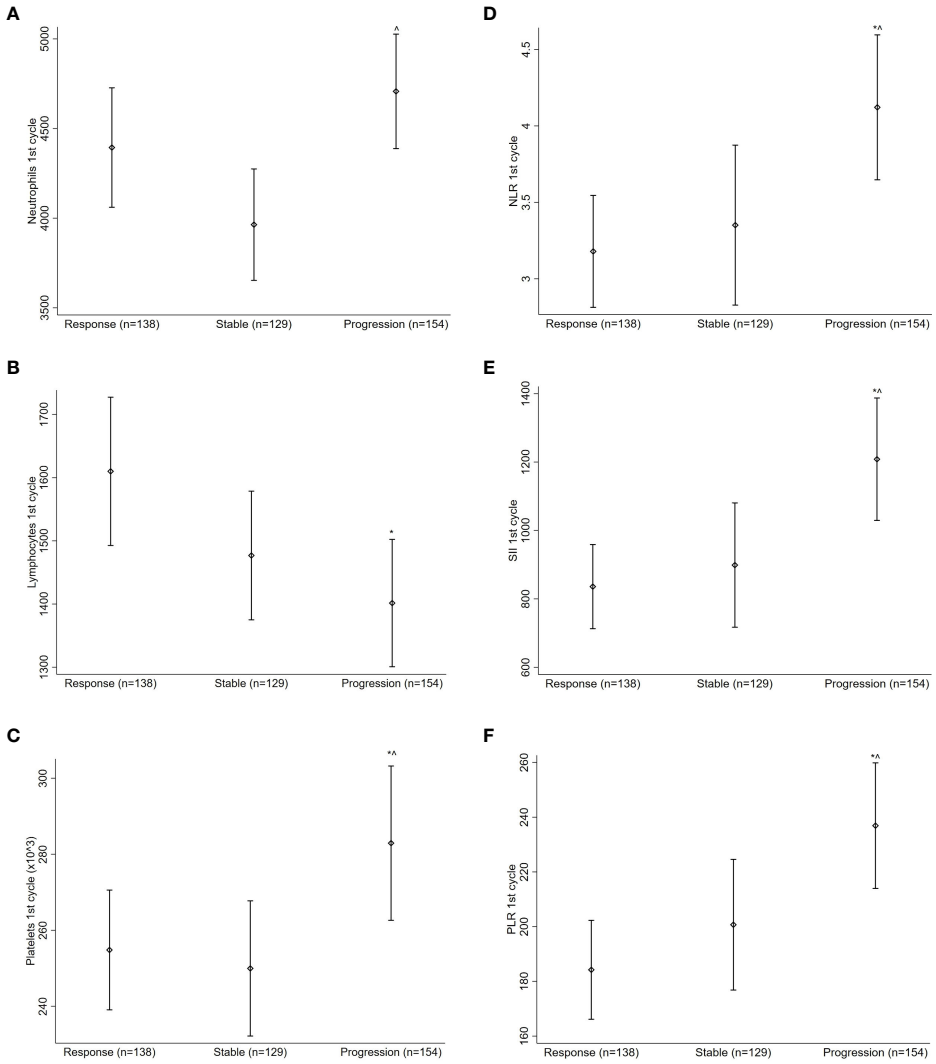

FIGURE 2
The baseline ACC and IR values according to the disease response to nivolumab.Neutrophils **(A)**, lymphocytes **(B)**, platelets **(C)**, NLR **(D)**, SII **(E)** and PLR **(F)** were assessed.*Significant differences compared with response (R); ^Significant difference compared with stable disease (S); 2A: p = 0.11 for S vs. R; p = 0.17 for progression (P) vs. R; p = 0.003 for P vs. S; 2B: p = 0.14 for S vs. R; p = 0.018 for P vs. R; p = 0.33 for P vs. S; 2C: p = 0.72 for S vs. R; p = 0.044 for P vs. R; p = 0.036 for P vs. S; 2D: p = 0.61 for S vs. R; p = 0.012 for P vs. R; p = 0.029 for P vs. S; 2E: p = 0.60 for S vs. R; p = 0.003 for P vs. R; p = 0.014 for P vs. S; 2F: p = 0.31 for S vs. R; p = 0.003 for P vs. R; p = 0.032 for P vs. S; p values were adjusted for multiple comparisons using the false discovery rate approach.

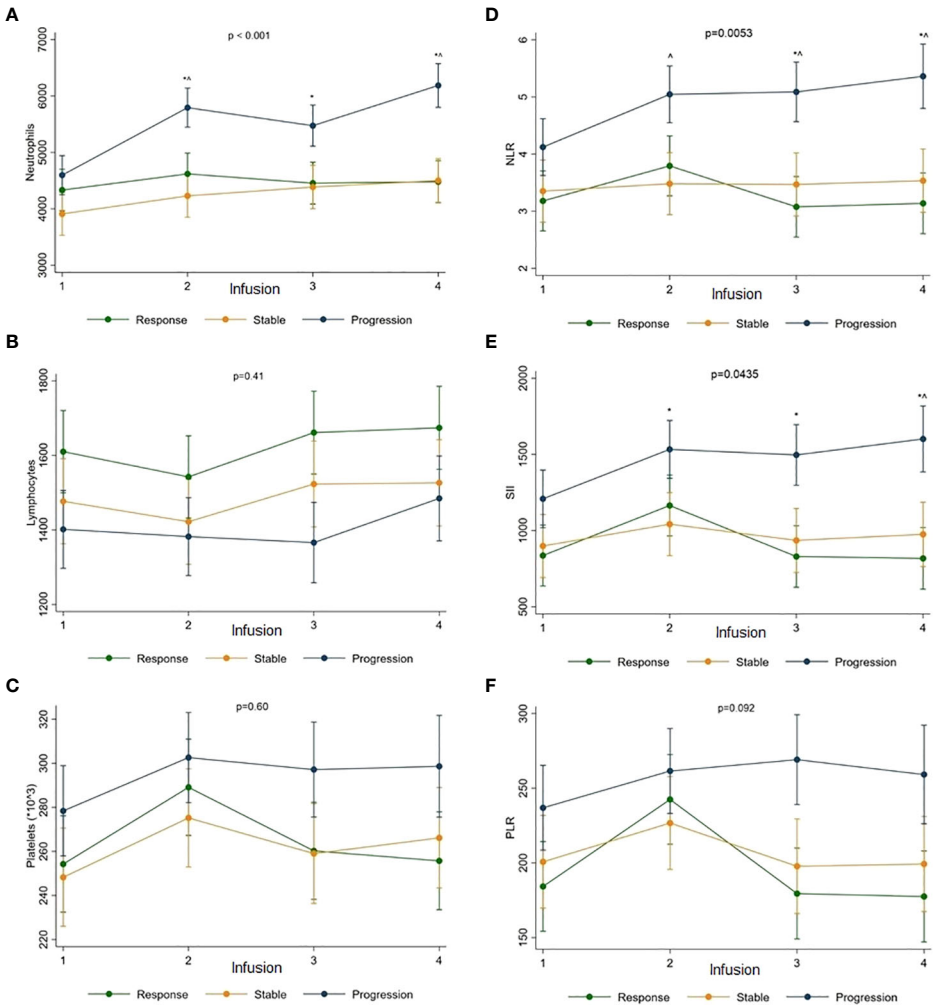

FIGURE 3
The ACC and IR value Δ according to the disease response to therapy.Neutrophils **(A)**, lymphocytes **(B)**, platelets **(C)**, NLR **(D)**, SII **(E)** and PLR **(F)** were assessed.*Significant differences compared with response; ^ Significant difference compared with stable disease.

off: ≥730x10e3/L) (p = 0.011), alongside IMDC intermediate (p < 0.001 with both models) and poor risk (p < 0.001 with both models) and the presence of bone metastases (p = 0.006 and p = 0.004, respectively) resulted as negative independent factors on OS at the multivariable analysis (Table 3).

Multivariable analysis results on PFS are reported in Table 1S and confirmed higher baseline NLR (p < 0.001) and SII (p = 0.038) values and increased early Δ of NLR (p = 0.001) and neutrophils (p = 0.023), alongside the IMDC intermediate- and poor-risk and the presence of bone metastases as negative prognostic factors (Table 1S).

The Harrel's c-index of the model with neutrophil early D was 0.692 for the OS and 0.630 for PFS, while with NLR early Δ was 0.693 and 0.644, respectively.

3.4.4 Interaction on survival outcomes between absolute cell counts and early Δ

A significant interaction between increased neutrophil early Δ (cut-off: ≥730x10e3/L) and higher baseline neuthrophil counts (cut-off: ≥4330x10e3/L) was found on PFS (p for interaction = 0.047) but not on OS (p for interaction = 0.12), with a longer median PFS for those patients with lower neutrophil early Δ (<730x10e3/L) and higher baseline neutrophil counts (≥4330x10e3/L) (HR = 1.76; 95% CI: 1.23-2.52; p = 0.002) (Figure 6S). No significant interactions between NLR early Δ (cut-off: ≥0.5) and baseline NLR values (cut-off: ≥3.2) were found in both PFS and OS (p for interaction = 0.36 and 0.89, respectively), suggesting that the association between NLR D, PFS, and OS was similar in patients with baseline NLR below or above the cut-off of 3.2 (Figure 7S).

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TABLE 2 Univariable analysis on survival outcomes of absolute cell counts and immune-inflammatory indices baseline and early Δ, and baseline clinical parameters.

Inflammatory indices	ROC-based cut-off values	PFS		OS	
		mPFS(95% CI)	Univariable(HR; 95% CI; p value)	mOS(95% CI)	Univariable(HR; 95% CI; *p* value
Absolute cell counts					
Baseline Neutrophils (x10e3/L)	≥ 4330	6.9 (5.1-10.9)	1.25; 0.99-1.56; p = 0.059	19.4 (12.6-26.4)	1.87; 1.40-2.49; **p < 0.001**
	< 4330	10.2 (8.4-14.3)	1.00 (ref)	NR	1.00 (ref)
Early Δ Neutrophils	≥ 730	6.1 (4.7-9.2)	1.29; 1.02-1.62; **p = 0.033**	20.8 (17.4-43.9)	1.34; 1.01-1.80; **p = 0.046**
	< 730	11.0 (9.3-13.9)	1.00 (ref)	46.9 (25.7-NR)	1.00 (ref)
Baseline Lymphocytes (x10e3/L)	< 1460	6.4 (5.0-8.4)	1.57; 1.25-1.98; **p < 0.001**	20.0 (17.1-27.7)	1.88; 1.39-2.53; **p < 0.001**
	≥ 1460	13.9 (9.9-18.5)	1.00 (ref)	NR	1.00 (ref)
Early Δ Lymphocytes	≥ -10	8.4 (5.5-12.1)	1.10; 0.88-1.38; p = 0.41	25.7 (20.1-43.9)	1.15; 0.86-1.54; p = 0.34
	< -10	9.9 (8.1-12.5)	1.00 (ref)	46.9 (23.0-NR)	1.00 (ref)
Baseline Platelets (x10e9/L)	≥ 263	8.4 (5.1-10.1)	1.40; 1.11-1.76; **p = 0.004**	19.4 (13.8-25.7)	1.92; 1.44-2.56; **p < 0.001**
	< 263	10.9 (7.8-15.0)	1.00 (ref)	NR	1.00 (ref)
Early Δ Platelets	≥ 17	8.5 (5.5-10.5)	1.07; 0.85-1.34; p = 0.56	26.4 (20.2-NR)	0.97; 0.73-1.30; p = 0.86
	< 17	10.8 (8.0-14.3)	1.00 (ref)	34.3 (20.8-NR)	1.00 (ref)
Indices					
Baseline NLR	≥ 3.2	5.8 (4.6-8.3)	1.58; 1.26-1.99; **p < 0.001**	18.7 (11.3-22.7)	2.10; 1.57-2.80; **p < 0.001**
	< 3.2	11.2 (9.5-16.6)	1.00 (ref)	NR	1.00 (ref)
Early Δ NLR	≥ 0.5	6.4 (5.0-9.3)	1.37; 1.09-1.72; **p = 0.007**	21.7 (18.4-43.9)	1.32; 0.99-1.76; p = 0.062
	< 0.5	12.1 (9.5-16.8)	1.00 (ref)	46.9 (25.7-NR)	1.00 (ref)
Baseline SII	≥ 720	6.1 (4.7-9.4)	1.51; 1.21-1.90; **p < 0.001**	18.7 (13.8-22.0)	2.27; 1.69-3.04; **p < 0.001**
	< 720	11.3 (9.5-18.3)	1.00 (ref)	NR	1.00 (ref)
Early Δ SII	≥ 218	6.4 (4.6-9.5)	1.24; 0.99-1.57; p = 0.061	24.5 (18.7-NR)	1.22; 0.91-1.64; p = 0.18
	< 218	11.0 (9.2-14.7)	1.00 (ref)	30.7 (23.7-NR)	1.00 (ref)
Baseline PLR	≥ 176	6.5 (4.7-9.5)	1.52; 1.21-1.91; **p < 0.001**	19.9 (15.5-22.7)	2.23; 1.66-3.01; **p < 0.001**
	< 176	11.5 (9.3-16.8)	1.00 (ref)	NR	1.00 (ref)
Early Δ PLR	≥ 21	9.2 (5.9-11.3)	1.07; 0.85-1.35; p = 0.54	27.7 (19.4-NR)	1.09; 0.82-1.45; p = 0.57
	< 21	9.9 (6.9-14.3)	1.00 (ref)	30.1 (21.7-NR)	1.00 (ref)
Baseline clinical parameter					

(Continued)

TABLE 2 Continued

Inflammatory indices	ROC-based cut-off values	PFS		OS	
		mPFS(95% CI)	Univariable(HR; 95% CI; p value)	mOS(95% CI)	Univariable(HR; 95% CI; *p* value
Heng score	Favorable	22.5 (16.4-35.2)	1.00 (ref)	NR	1.00 (ref)
	Intermediate	8.2 (5.9-9.5)	1.85; 1.36-2.51; **p < 0.001**	25.7 (20.1-34.3)	2.83; 1.79-4.50; **p < 0.001**
	Poor	2.9 (2.2-5.5)	3.28; 2.16-4.99; **p < 0.001**	8.1 (3.7-10.7)	7.13; 4.12-12.37; **p < 0.001**
Metastatic at diagnosis	Yes	6.4 (5.3-9.3)	1.21; 0.96-1.53; p = 0.11	21.7 (17.5-34.3)	1.40; 1.05-1.87; **p = 0.023**
	No	11.2 (9.3-14.7)	1.00 (ref)	46.9 (24.8-NR)	1.00 (ref)
Nephrectomy	Yes	9.9 (8.3-12.5)	0.60; 0.42-0.85; **p = 0.004**	43.9 (25.7-NR)	0.43; 0.29-0.62; **p < 0.001**
	No	4.0 (2.9-8.8)	1.00 (ref)	14.5 (8.6-19.4)	1.00 (ref)
Histologic subtype	Clear-cell	9.5 (7.9-11.5)	0.95; 0.69-1.31; p = 0.77	29.5 (22.0-NR)	1.08; 0.71-1.63; p = 0.72
	Non-clear cell	6.6 (5.0-13.6)	1.00 (ref)	NR	1.00 (ref)
Lymph node metastases	Yes	7.4 (5.6-10.1)	1.15; 0.92-1.45; p = 0.22	25.7 (19.9-30.7)	1.28; 0.95-1.71; p = 0.10
	No	11.0 (8.8-13.8)	1.00 (ref)	46.9 (22.7-NR)	1.00 (ref)
Viscera metastases	Yes	9.3 (6.9-11.1)	1.09; 0.72-1.64; p = 0.69	29.8 (22.0-NR)	1.04; 0.62-1.74; p = 0.88
	No	11.3 (5.8-23.4)	1.00 (ref)	25.7 (16.7-NR)	1.00 (ref)
Bone metastases	Yes	6.4 (4.6-8.4)	1.51; 1.20-1.91; **p = 0.001**	18.7 (13.1-25.0)	1.81; 1.36-2.42; **p < 0.001**
	No	11.3 (9.3-16.0)	1.00 (ref)	46.9 (29.8-NR)	1.00 (ref)
Line of therapy	2	9.5 (6.6-12.1)	1.00 (ref)	30.1 (21.4-NR)	1.00 (ref)
	3	9.5 (6.1-13.1)	1.06; 0.84-1.35; p = 0.61	NR	0.97; 0.71-1.31; p = 0.83
	>4	8.3 (3.2-16.6)	0.94; 0.68-1.28; p = 0.68	18.1 (9.3-NR)	0.86; 0.57-1.30; p = 0.48

Early Δ value variations between second and first therapy infusion, mOS median overall survival, mPFS median progression-free survival, NLR neutrophils-to-lymphocytes ratio, NR not reached, PLR platelets-to-lymphocytes ratio, ROC receiving operating curve, SII systemic immune-inflammatory index.
In bold, significant p-values.

4 Discussion

In the era of tyrosine kinase inhibitors (TKIs), ICIs and their combinations for mRCC, baseline clinical, and laboratory characteristics of patients incorporated into the IMDC score (37, 38) still represent the critical factors clinicians consider for the treatment decision making (2, 3, 39). More recently, we proposed implementing the IMDC prognostic stratification by the Meet-URO score, which was demonstrated in large series (34, 40) to be more accurate than IMDC alone by two additional independent prognostic factors (the presence of bone metastases and the NLR). Tumor biomarkers, like the PD-L1 expression or the TMB, have not showed yet a clinical utility, particularly for the ICIs (8, 9), nor dynamic biomarkers, whose variations during treatment might early indicate the tumor sensitivity or resistance, are available. Moreover, early predictors of disease progression could spare patients from ineffective treatments and their related toxicity and could potentially improve patients' outcomes by allowing an earlier change of treatment line (41).

The early variations of inflammatory indices from peripheral blood are captivating dynamic biomarkers as they have consistently shown their prognostic value in several tumor

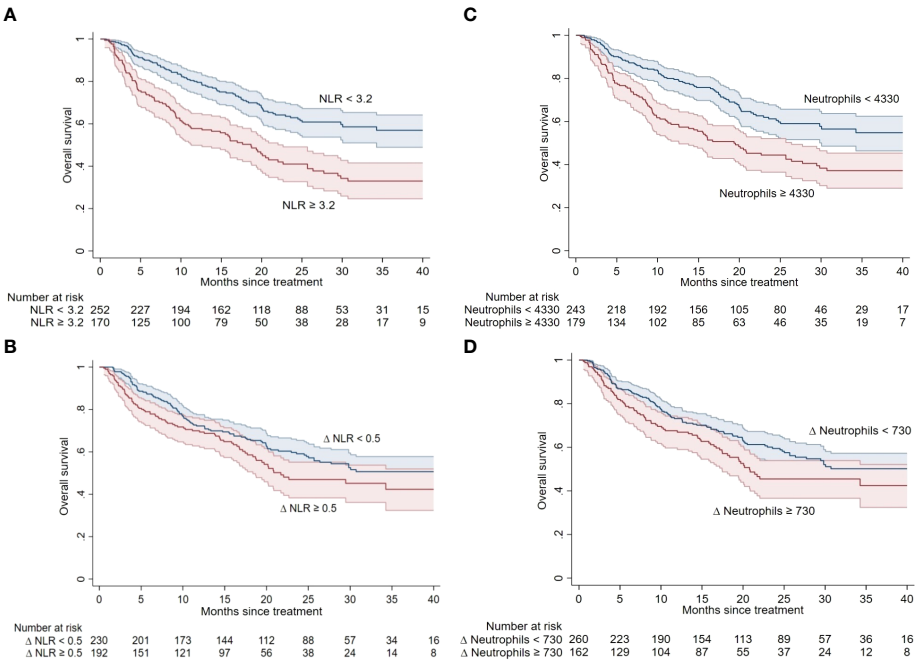

FIGURE 4
The univariable analyses of baseline and early Δ of NLR **(A, B)** and neutrophils **(C, D)**.

types and treatment settings in addition to their easy and relatively inexpensive assessment and reproducibility in clinical practice (21, 22, 34). Evidence is accumulating regarding the prognostic value of their early variations, mainly involving the NLR, in advanced non-small-cell lung cancer (23, 25, 29–31), small-cell lung cancer (33), esophageal squamous cell carcinoma (32), and mRCC treated with ICIs (24, 28). However, the mechanisms underlying the dynamic variations of inflammatory indices from peripheral blood during treatments and whether they reflect a change in the immunological status in response to treatment, especially to ICIs, are still unclear.

On these premises, the results of this pre-specified secondary analysis of the Meet-URO 15 study (34), focusing on the quantitative variations of cellular counterparts of the mainly used IR (or the NLR, SII and PLR), provided us with the following four key observations. Firstly, during the initial treatment with nivolumab, there was a consistent neutrophil and relative NLR increase. Secondly, patients with higher platelet and lower lymphocyte baseline counts, and increasing neutrophil counts during the ICI, were more likely to develop disease progression than response. This may also explain why all the baseline IR values but only increasing NLR and SII (i.e., not the PLR) were predictive of PD. Thirdly, survival outcomes (both OS and PFS) were worse for patients with baseline lower lymphocytes and higher platelets (and consequently higher NLR, SII and PLR), and early neutrophil increase over treatment. The latter was particularly relevant in patients with higher baseline neutrophils. Finally, besides baseline NLR and the other known prognostic variables, early rise in neutrophils and NLR resulted as independent prognostic factors on both OS and PFS.

Increased peripheral neutrophils promote tumor development, invasiveness, metastasis, and resistance to treatment (42). The intra-tumoral neutrophil count is also directly related to blood neutrophils (43). Blood lymphocyte counts are associated with the immunological response to malignancy. As a result, the body's capacity to inhibit cancer cells may be impacted when inflammation results in prolonged lymphocytopenia, including CD4+ and CD8+ T lymphocytes (42, 44). The contribution of lymphocytes from peripheral blood, and their early increase, to the tumor response in mRCC patients treated with immunotherapy was already demonstrated with interleukin-2 treatment (45). Platelets promote an immunosuppressive tumor microenvironment (TME) in addition to tumor-induced aggregation and clotting by secreting angiogenic and mitogenic growth factors and immunosuppressive cytokines and physically shielding tumor cells from cytotoxic lymphocytes and natural killer (NK) cells invading the tumor (46). In addition, they recruit leukocytes to tumor sites and regulate responses of the adaptive immune system (47). NLR may work as a stand-in for tumor inflammation and most likely reflects the suppression of T-cell proliferation by myeloid-derived suppressor cells (MDSC) (48).

The current analysis could not assess the predictive role for immunotherapy of baseline levels or dynamics of peripheral-

TABLE 3 Multivariable analysis on OS of absolute cell counts and immune-inflammatory indices baseline and early Δ, and baseline clinical parameters.

Inflammatory indices	ROC-based cut-off values	Multivariable Cox regression for OS	
		NLR (HR; 95% CI; *p* value)	Neutrophils (HR; 95% CI; p value)
Baseline NLR	≥ 3.2	1.83; 1.35-2.49; **p < 0.001**	
	< 3.2	1.00 (ref)	
Early Δ NLR	≥ 0.5	1.46; 1.08-1.96; **p = 0.014**	
	< 0.5	1.00 (ref)	
		p value for interaction baseline NLR and ΔNLR = 0.73	
Baseline Neutrophils	≥ 4330 x10e3/L		1.82; 1.35-2.45; **p < 0.001**
	< 4330 x10e3/L		1.00 (ref)
Early Δ Neutrophils	≥ 730 x10e3/L		1.48; 1.09-1.99; **p = 0.011**
	< 730 x10e3/L		1.00 (ref)
			p value for interaction baseline Neutrophils and ΔNeutrophils = 0.074
Clinical parameter			
IMDC score	Favorable	1.00 (ref)	1.00 (ref)
	Intermediate	2.79; 1.73-4.50; **p < 0.001**	2.68; 1.66-4.32; **p < 0.001**
	Poor	5.46; 3.03-9.82; **p < 0.001**	5.52; 3.07-9.93; **p < 0.001**
Metastatic at diagnosis	Yes	0.85; 0.61-1.18; p = 0.32	0.84; 0.60-1.17; p = 0.30
	No	1.00 (ref)	1.00 (ref)
Nephrectomy	Yes	0.67; 0.43-1.04; p = 0.077	0.57; 0.37-0.87; **p = 0.009**
	No	1.00 (ref)	1.00 (ref)
Bone	Yes	1.52; 1.13-2.04; **p = 0.006**	1.55; 1.15-2.08; **p = 0.004**
	No	1.00 (ref)	1.00 (ref)

CI confidence interval, early Δ value variations between 2nd and 1st therapy infusion, HR hazard ratio, IMDC International Metastatic RCC Database Consortium Risk Score for RCC, NLR neutrophils-to-lymphocytes ratio, OS overall survival, RCC renal cell carcinoma, ROC receiving operating curve.
In bold, significant p-values.

blood parameters based on neutrophil, lymphocyte, and platelet absolute cell counts, particularly regarding their potential correlation with TME or whether they corresponded to the intratumoral immune response modifications favored by ICIs. For those issues, we should have had a TME correlate and a control arm. Thus, we cannot provide a mechanistic link between the different immune-inflammatory cell populations in the peripheral blood and TME. Moreover, it was not the scope for the current analysis, which focused on the only prognostic value of those blood baseline and dynamic peripheral-blood immune or inflammatory cells and their derived ratios based on their association with survival outcomes of patients with mRCC following immunotherapy. Nonetheless, we believe the findings retain a relevant clinical utility for their exclusive prognostic value while hypothesis-generating for future translational, correlative, or comparative studies. For instance, their routine assessment could represent a helpful tool to predict treatment resistance early. In fact, outside clinical trials, the first radiological disease evaluation is rarely performed earlier than 3 months after the treatment start. Thus, the early increase of neutrophils and NLR, just at the second ICI administration, might prompt the clinician to anticipate the radiological reassessment, thus saving toxicity to patients and the health system and offering the patient a different treatment before clinical worsening would make it not possible, or informing novel prospective adaptive studies with arm allocation based on treatment response (49, 50). Notably, before ICIs and their combinations were used as the first-line treatment, only 42%–57% of mRCC patients were estimated to receive a second-line therapy, and this proportion might have not dramatically increased (51, 52).

We acknowledge as study limitations the retrospective data and analysis (including missing clinical information interplaying with ACC and IR, like comorbidity and steroids, or other concomitant drugs), the possible selection bias (as enrolled patients had to receive at least two nivolumab administrations), the variable timing and clinician-lead disease reassessment (which might have impacted on the definition of disease response), the restriction to variations of ACC as components of the IR (i.e., albumin, lactate dehydrogenase, C-reactive protein, and other inflammatory parameters were not considered), which make more important an external validation of our findings. Another relevant study limitation is the disused

treatment setting for immunotherapy. However, the proof-of-principle value of the analysis may be retained. Baseline values and early variations of peripheral blood inflammatory ratios and their cellular components were associated with the clinical outcomes of pretreated patients with metastatic renal cell carcinoma receiving single-agent immunotherapy. It needs confirmation in the front-line setting with immunotherapy-based combinations for which we planned *ad hoc* analyses. Immortal and lead time biases are further analysis limitations related to the variation of blood inflammatory ratios and their dynamic assessment. However, we had a relatively low proportion (7.4% of patients) who did not reach the second treatment cycle, and most patients were treated in the second-line setting. Regarding the immortal time bias, early deaths due to disease progression would be expected in patients with high delta values of blood inflammatory ratios, thus not changing the observed effect direction. Furthermore, the late dynamics of ACC and IRR and their association were not investigated.

Nevertheless, this study is one of the largest reports on the dynamics of inflammatory indices from peripheral blood during treatment with ICIs. It adds biological insights to the prognostic value of IR based on the different baseline and early value variations of their specific cellular components. Moreover, it pointed out the early variation of neutrophils and NLR as new prognostic factors with clinical utility for on-treatment decisions, thus offering a new dynamic non-invasive, routinely available tool, at no additional costs, to help clinicians with early on-treatment decisions concerning patients with mRCC treated with ICIs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Regional ethical committee of Liguria - registration number 068/2019. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study concept and design, SR, AS, MSt, GB, GF, and SB; GB and GF contributed equally as senior authors; SR, AS, and MSt contributed equally as first authors. Acquisition and curation of data, all authors; statistical analysis, AS; interpretation of data, SR, AS, MSt, GB, GF, and SB; drafting of the manuscript, SR, AS, MSt, and GB; critical revision of the manuscript for important intellectual content: SR, GB, GF, SB, and DS; supervision, SR, GB, and GF. All authors have read and agree to the published version of the manuscript.

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Conflict of interest

SR received honoraria as a speaker at scientific events and travel accommodation from Amgen, GSK, BMS, and MSD. GB reports personal fees from AstraZeneca, Janssen-Cilag, Boehringer Ingelheim, Roche, and non-financial support from BMS, AstraZeneca, MedImmune, Pierre Fabre, and IPSEN. GF services advisory boards for Astellas, Janssen, Pfizer, Bayer, MSD, and Merck and received travel accommodation from Astellas, Janssen, and Bayer. SB received honoraria as speaker at scientific events and advisory role by BMS, Pfizer, MSD, Ipsen, Roche, Eli Lilly, AstraZeneca, Pierre-Fabre, and Novartis. DS received honoraria for the advisory board from Amgen, Jansen, MSD, BMS, Bayer, Astra Zeneca, Ipsen, Novartis, and Merck. UG serves as advisory/board member of Astellas, Bayer, BMS, IPSEN, Janssen, Merck, Pfizer, and Sanofi, and received research grant/funding to the institution from AstraZeneca, Roche, Sanofi and travel/accommodations/expenses from BMS, IPSEN, Janssen, and Pfizer. PZ services advisory boards/consulting for Pfizer, BMS, MSD, IPSEN, Novartis, Roche, Amgen, AstraZeneca, Sanofi, Janssen, and Astellas. GPro received a personal fee for consulting or advisory role AstraZeneca, Bayer, BMS, Eisai, Janssen, Ipsen, Merck, MSD, Novartis, and Pfizer and a research grant from Astellas, Ipsen, Novartis. MSo received honoraria as consultant or advisory role from Janssen; grants for participation at scientific events from Ipsen, Janssen, Bristol Myers Squibb, Pfizer, Astellas Pharma, Sanofi, Roche, and Novartis; and research funding from Roche, Merck, Janssen. ACo receives speaker fees/grant consultancies from Astrazeneca, BMS, MSD, Roche, Novartis, and Astellas. FMo received grants from MSD and Pfizer. GR received honoraria for advisory

boards or invited speaker fees from BMS, Astellas, Bayer, Ipsen, Novartis, Roche, and AstraZeneca.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.955501/full#supplementary-material

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Clinical implications of the tumor microenvironment using multiplexed immunohistochemistry in patients with advanced or metastatic renal cell carcinoma treated with nivolumab plus ipilimumab

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Purpose: Immune checkpoint inhibitors (ICIs) such as nivolumab and ipilimumab (N/I) are important treatment options for advanced renal cell carcinoma (RCC). The tumor microenvironment (TME) in these ICI-treated patients is largely unknown.

Methods: Twenty-four patients treated with N/I between July 2015 and June 2020 were analyzed. Multiplexed immunohistochemistry (mIHC) was conducted to define the TME, including various T cell subsets, B cells, macrophages, and dendritic cells.

Results: The median age of the study patients was 61 years (range, 39–80) and 75.0% of these cases were men. The objective response rate with N/I was 50.0%. The densities of the CD8+ cytotoxic T cells (P=0.005), specifically CD137+ CD8+ T cells (P=0.017), Foxp3- CD4+ helper T cells (P=0.003), Foxp3+ CD4+ regulatory T cells (P=0.045), CD68+ CD206- M1 macrophages (P=0.008), and CD68+ CD206+ M2 macrophages (P=0.021) were significantly higher in the treatment responders. At a median follow-up duration of 24.7 months, the median progression-free survival (PFS) was 11.6 months. The high densities (≥median) of Foxp3- CD4+ helper T cells (P=0.016) and CD68+ CD206- M1 macrophages (P=0.008) were significantly associated with better PFS, and the density of CD137+ CD8+ cytotoxic T cells (P=0.079)

was marginally associated with better PFS. After multivariate analysis, the higher density of Foxp3- CD4+ helper T cells was independently associated with better PFS (hazard ratio 0.19; P=0.016).

Conclusion: The properties and clinical implications of the TME properties in RCC indicate that Foxp3- CD4+ helper T cells, M1 macrophages, and CD137+ CD8+ T cells are potential predictive biomarkers and treatment targets.

KEYWORDS

renal cell carcinoma, tumor microenvironment, immune checkpoint inhibitors, response, survival

Introduction

The prognosis of advanced renal cell carcinoma (RCC) has considerably improved in recent decades due to the introduction of immune checkpoint inhibitors (ICIs), which block programmed death (ligand) 1 (PD-L1) and cytotoxic T lymphocyte antigen-4 (CTLA4) and combinations of ICI plus vascular endothelial growth factor receptor tyrosine kinase inhibitor. Following the phase III CheckMate-214 trial, a first-line therapy with nivolumab (an anti-PD-1 inhibitor) plus ipilimumab (an anti-CTLA4 inhibitor), compared to sunitinib alone, was found to improve the objective response rate (ORR) (42% vs. 27%, P<0.001) and overall survival (OS) (hazard ratio [HR] 0.63, P<0.001) in intermediate- and poor-risk patients (1). The long-term follow-up analysis of these trial subjects also demonstrated durable efficacy benefits with nivolumab plus ipilimumab compared with sunitinib (2, 3). However, only a limited number of patients benefit from ICIs. The ORR was 42% with nivolumab plus ipilimumab versus 27% with sunitinib (P<0.001). Approximately 20% (83/425) of the intermediate- and poor-risk patients from the CheckMate-214 trial (1) experienced initial disease progression and had relatively short progression-free survival (PFS).

There are currently no validated biomarkers for predicting the ICI treatment response. The predictive and prognostic significance of PD-L1 expression, genomic mutations, the tumor mutation burden, and gene expression patterns have previously been explored in ICI-treated patients (4–7). The peripheral blood markers such as absolute neutrophil, lymphocyte, monocyte, eosinophil, and immune cell counts have been also investigated for the prediction of response to ICI treatment (8–10). However, understanding the determinants of these treatment responses is challenging. Given that the tumor microenvironment (TME) can influence the response to ICIs, an investigation of its heterogeneous characteristics is necessary to predict this response, and a better understanding of the underlying immunity in the patients could suggest novel strategies to further improve clinical outcomes (11, 12).

Among various immune subsets in TME, T cell subsets such as cytotoxic CD8+ T cells, helper CD4+ T cells, and regulatory CD4+ T cells are recognized as key components in the anti-tumor immune response (13–15). CD8+ T cells are activated through the CD137 signaling, thereby enhancing T cell survival and promoting their effector function (16). Macrophages, dendritic cells, and B cells also participate in antigen presentation, inflammation, and anti-tumor activity (17). Previous studies have examined the prognostic value of various immune subsets using conventional immunohistochemistry (IHC) in various cancer (18, 19). However, conventional IHC has limitations in that it is impossible to stain multiple markers at once on the same specimen slide to evaluate immune subsets and cannot evaluate immune cell counts. The multiplexed IHC (mIHC) is the quantitative multispectral imaging method that can discriminate immune subsets based on the expression of multiple markers. This novel method has been validated to reflect conventional IHC-based immune cell evaluation and is increasingly used to assess the immune profiles of the TME (20, 21).

In our present study, we performed mIHC to investigate the features of TME in patients with advanced RCC receiving nivolumab plus ipilimumab and evaluated the prognostic implications for the prediction of a treatment response.

Materials and methods

Patients

A total of 24 patients with advanced or metastatic RCC were treated with nivolumab plus ipilimumab as first-line therapy at Asan Medical Center, Seoul, Republic of Korea, between July 2015 and June 2020. mIHC was retrospectively performed to investigate the characteristics of TME in these patients. This

retrospective study was approved by the Institutional Review Board of Asan Medical Center (study number: 2019-1712), and it was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Patients with International Metastatic RCC Database (IMDC) (22) at intermediate- or poor-risk received nivolumab (3 mg/kg) and ipilimumab (1 mg/kg) intravenously as a first-line therapy every 3 weeks in four doses, followed by nivolumab (3 mg/kg) every 2 weeks. The tumor response was assessed using computed tomography every 6 to 9 weeks for the first year and then every 9 to 12 weeks thereafter until disease progression or discontinuation of ICI treatment, based on the response evaluation criteria in solid tumors (RECIST) criteria v1.1 (23).

Multiplexed immunohistochemistry

Optimized fluorescent mIHC was performed by tyramide signal amplification (TSA) using a Leica Bond Rx™ Automated Stainer (Leica Biosystems, Newcastle, UK). Cells were stained with antibodies against CD20 (ab9475; Abcam, Cambridge, UK), CD4 (ab133616; Abcam), CD103 (ab129202; Abcam), Foxp3 (ab20034; Abcam), CD137 (ab197942; Abcam), CD8 (MCA1817; Bio-Rad, Hercules, CA, USA), CD206 (NBP1-90020; Novus Biologicals, Littleton, CO, USA), CD68 (ab 192847; Abcam), CD11c (ab52632; Abcam), MHCII (ab 7856; Abcam), and PD-L1 (13684S; Cell Signaling Technology, Danvers, MA, USA). The fluorescence signals were captured with the following fluorophores: Opal 480, Opal 520, Opal 570, Opal 620, Opal 690, and Opal 780. Multiplex-stained slides were obtained using the Vectra® Polaris Quantitative Pathology Imaging System (PerkinElmer, Boston, MA, USA). The images were analyzed using inForm 2.4.4 image analysis software (PerkinElmer) and Spotfire™ software (TIBCO Software Inc., Palo Alto, CA, USA).

Regions of interest (ROIs) representing each tissue specimen were carefully chosen by pathologists, based on hematoxylin and eosin slides, and approximately 7–11 ROIs were thereby selected for each tissue specimen. We also subdivided the tumor into center, margin, and stroma regions in the available tissues from surgical specimens. The immune cell activity and its clinical value may be different according to the spatial distribution. Representative images are shown in Figure 1, and the implications for each marker are explained in Table S1. CD8+ was used for indicating cytotoxic T cells; CD103+ CD8+ for tissue-resident T cells and CD137+ CD8+ or CD137+ CD4+ for costimulatory 4-1BB-expressing T cells, both used as activated T cells; Foxp3- CD4+ for helper T cells; Foxp3+ CD4+ for regulatory T cells; CD20+ for B cells; CD206- CD68+ for M1-polarized macrophages; CD206+ CD68+ for M2-polarized macrophages; CD11c+ MHC class II+ for antigen-presenting dendritic cells; and PD-L1+ for immune regulatory molecules. Cell densities are measured as the mean/mm^2 for each cell population.

Statistical analyses

Categorical and quantitative data were compared using the chi-square test or Fisher's exact test, and Mann–Whitney U tests. The mean levels of the markers among the three groups were compared using analysis of variance (ANOVA). Multiple comparison tests were not performed. The PFS was calculated from the date of ICI initiation to the date of disease progression or death from any cause, whichever occurred first. The OS was calculated from the date of ICI initiation to the date of death from any cause. Survival was estimated using the Kaplan–Meier method, and the log-rank test was used to compare the differences between the curves. A two-sided *P*-value <0.05 was considered significant, and all statistical analyses were performed using the statistical package for the social sciences (SPSS) 25.0 software package (IBM SPSS Statistics, Chicago, IL, USA).

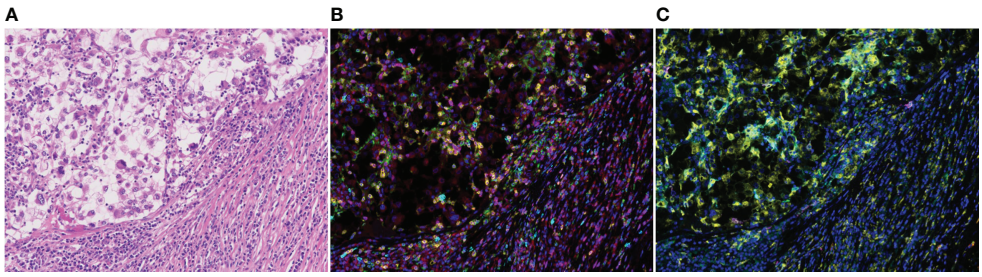

FIGURE 1
Representative examples of multiplexed immunohistochemical staining of advanced renal cell carcinoma tissue sections. **(A)** hematoxylin and eosin staining. **(B)** CD20, CD4, CD103, Foxp3, CD137, and CD8. **(C)** CD206, CD68, CD11c, MHCII, and PDL1. Original magnification, x 200.

Results

Patient characteristics

A total of 24 patients underwent mIHC analysis in this study. The baseline patient characteristics are summarized in Table 1. The median patient age was 61 years (range, 39–80 years), and 75.0% were men. The available tissues were obtained prior to nivolumab plus ipilimumab treatment. Tissues were obtained from surgery (n=16) or biopsy (n=8).

Table 1 summarizes the efficacy of the ICI treatments. The ORR and disease control rate (DCR) were 50.0% and 70.8%, respectively. At a median follow-up duration of 24.7 months (95% confidence interval [CI], 21.5–28.0), 14 patients (58.3%) experienced disease progression and the median PFS was 11.6 (95% CI, 5.2–17.9) months. The median OS was not reached because only five (20.8%) patients had died at the time of the analysis.

TABLE 1 Baseline characteristics of the study patients and clinical outcomes with nivolumab plus ipilimumab.

	Total patients (n=24, %)
Median age, years (range)	61 (39–80)
Sex	
male	18 (75.0)
female	6 (25.0)
IMDC risk group	
Intermediate	14 (58.3)
Poor	10 (41.7)
Histology type	
Clear cell*	23 (95.8)
Presence of sarcomatoid component	8 (33.3)
Site of metastasis	
Lymph node	10 (41.7)
Lung	19 (79.2)
Liver	3 (12.5)
Bone	10 (41.7)
Previous nephrectomy	17 (70.8)
Response and survival with nivolumab plus ipilimumab	
Complete response	3 (12.5)
Partial response	9 (37.5)
Stable disease	5 (20.8)
Progressive disease	7 (29.2)
Objective response rate	12 (50.0)
Disease control rate	17 (70.8)
Median progression-free survival	11.6 (95% CI 5.2–17.9) months
Median overall survival	Not reached

IMDC; International Metastatic RCC Database Consortium, CI; confidence interval
*One patient had a sarcomatoid renal cell carcinoma.

Association of tumor microenvironment immune cells with responses to nivolumab plus ipilimumab

The densities of the T cell subsets, B cells, macrophages, dendritic cells, and PD-L1-expressing immune cells were compared between responders (complete response [CR] + partial response [PR]) and non-responders. The density of immune cells in the TME of the advanced RCC lesions is listed according to the response in Table 2. The density of CD8+ cytotoxic T cells (P=0.005), Foxp3- CD4+ helper T cells (P=0.003), and Foxp3+ CD4+ regulatory T cells (P=0.045) was significantly higher in responders than in non-responders. Specifically, CD137+ CD8+ T cells (P=0.017) was highly infiltrated in the responders. A high infiltration of CD68+ CD206- M1 macrophages or CD68+ CD206+ M2 macrophages was significantly associated with achieving a response to nivolumab plus ipilimumab (P=0.008 and P=0.021). Otherwise, there were no significant differences in the density of CD11c+ MHC class II+ dendritic cells or PD-L1-expressing immune cells between the responders and non-responders.

Association of tumor microenvironment immune cells with progression-free survival

Each TME marker was classified into high (≥median) and low (<median) groups. The high density of Foxp3- CD4+ helper T cells (P=0.016) and CD68+ CD206- M1 macrophages (P=0.008) was significantly associated with better PFS (Figures 2A, B). The high density of CD137+ CD8+ cytotoxic T cells (P=0.079), CD137+ CD4+ cytotoxic T cells (P=0.126), and CD20+ B cells (P=0.185) was marginally associated with better PFS (Figures 2C–E). Multivariate analysis revealed that the higher density of Foxp3- CD4+ helper T cells was independently associated with better PFS (hazard ratio 0.19, 95% CI 0.05-0.73; P=0.016) (Table 3). There were no significant differences in the PFS according to the densities of CD11c+ MHC class II+ dendritic cells or PD-L1-expressing immune cells.

Spatial distribution of tumor microenvironment immune cells

To quantify the infiltration of immune cell subsets, associated with the efficacy with nivolumab plus ipilimumab, according to their spatial distribution, the tumor regions were subdivided into a center, margin, and stroma in the available tissues (n=14). The density of FoxP3- CD4+ helper T cells, CD137+ CD8+ cytotoxic T cells, and CD137+ CD4+ T cells

TABLE 2 Immune cell infiltration densities between the treatment responders and non-responders.

	Nivolumab plus ipilimumab (n=24, %)		
	Responders (n=12), median (IQR 25%-75%)	Non-responders (n=12), median (IQR 25%-75%)	*P*-value
CD8+ cytotoxic T cells	394.2 (157.7-670.4)	98.1 (50.4-279.3)	0.005
CD103+ CD8+ tissue-resident T cells	18.2 (2.5-33.1)	8.3 (3.7-15.5)	0.148
CD137+ CD8+ T cells	5.6 (1.9-45.7)	0.9 (0.0-8.3)	0.017
Foxp3- CD4+ helper T cells	349.1 (251.2-799.6)	58.2 (25.3-147.2)	0.003
Foxp3+ CD4+ regulatory T cells	15.8 (2.3-22.6)	0.7 (0.2-3.1)	0.045
CD137+ CD4+ T cells	7.0 (2.2-120.9)	3.3 (0.0-33.5)	0.090
CD20+ B cells	21.1 (5.8-40.7)	3.3 (0.7-31.5)	0.134
CD68+ CD206- M1 macrophages	643.67 (408.95-1148.24)	126.50 (71.59-575.16)	0.008
CD68+ CD206+ M2 macrophages	3.67 (1.10-12.46)	0.63 (0.0-2.42)	0.021
CD11c+ MHC class II+ dendritic cells	0 (0-1.4)	0 (0-0)	0.557
PD-L1+ cells	770.6 (506.9-1417.7)	388.3 (92.4-1143.2)	0.223

IQR, interquartile.
Cell densities are measured as the mean/mm^2 for each cell population.

seemed to be numerically higher in the tumor margin than in the stroma or center (Figure S1).

Association of tumor microenvironment immune cells with treatment-related adverse event to nivolumab plus ipilimumab

Treatment-related adverse event (TRAE) occurred in 16 (66.7%) (Table S2). The most common TRAE of any grade was rash (n=8, 33.3%) and there were grade 3 hyperglycemia (n=4, 16.7%). Common TRAE (>10%) of any grade included ALT elevation (n=7, 29.2%), AST elevation (n=5, 20.8%), anorexia (n=5, 20.8%), diarrhea (n=4, 16.7%), pruritus (n=4, 16.7%), and fatigue (n=3, 12.5%). Most of them were in grade 1. There were no significant differences in immune cell densities between patients with any grade of TRAE and those without any TRAE (Table S3), and patients with grade 3 hyperglyceima and those without grade ≥ 3 TRAE (Table S4).

Discussion

The current study showed a significant association between the TME in RCC patients and the response and PFS to nivolumab plus ipilimumab treatment through mIHC analysis. Notably, the higher density of Foxp3- CD4+ helper T cells and CD68+ CD206- M1 macrophages was significantly associated with both the treatment response and better PFS, respectively. The density of Foxp3- CD4+ helper T cells remained a significant factor in terms of the PFS after multivariate analysis.

There is growing interest in unraveling the role of TME in identifying biomarkers but exploring its heterogeneity is a complex task in highly immune-infiltrated RCC (11, 12). A simple measurement of CD8+ T cells is unlikely to be predictive of an ICI response (11), and a defective T cell function in RCC has been reported in several studies (24–26). Emerging evidence has suggested that CD4+ T cells may also play a critical role in immune responses. Foxp3- CD4+ helper T cells have been shown to promote the priming of tumor-specific CD8+ T cells and help elicit durable T cell responses by interacting with dendritic cells in an MHCII-dependent manner (14). CD68+ CD206- M1 macrophages participate in antigen presentation, inflammation, and anti-tumor activity (27). We found also in our current analyses that CD137+ CD8+ T cells, as a population of activated T lymphocytes, had a significantly higher level of infiltration in the responders compared with the non-responders, and that this higher density was marginally associated with better PFS. It is well known that signaling through CD137 induces the activation of CD8+ T cells, thereby enhancing T cell survival, promoting their effector function, and favoring memory differentiation (28). Regarding the Foxp3+ CD4+ regulatory T cells known to have opposing roles in antitumor immunity (14), we found in our present analyses that the density of Foxp3+ CD4+ regulatory T cells was inversely higher in responders than in non-responders. This may be explained by the fact that the antitumor activity of anti-CTLA4 inhibitors is dependent on the depletion of CTLA4-expressing regulatory T cells in the TME through antibody-dependent cellular cytotoxicity (29). Hence, patients with a higher density of Foxp3+ CD4+ regulatory T cells can be more susceptible to anti-CTLA4 inhibitors. It has been reported in this regard that a higher Foxp3+ CD4+ regulatory T cell level at

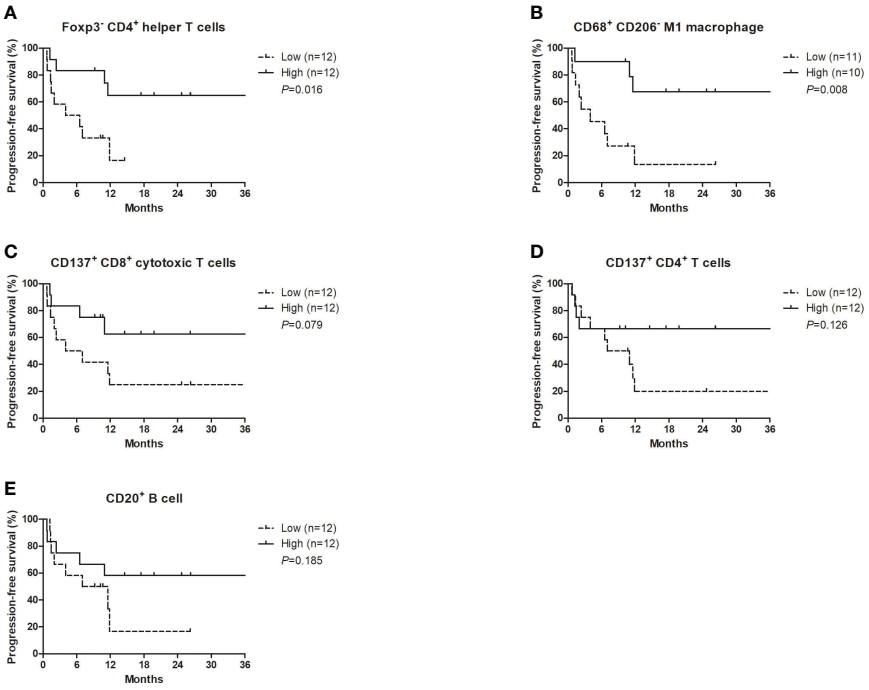

FIGURE 2
Progression-free survival with nivolumab plus ipilimumab according to the densities of certain T cell subsets, $CD20^+$ B cells, and M1 macrophages at the tumor margin. **(A)** $Foxp3^-$ $CD4^+$ helper T cells, **(B)** $CD68^+$ $CD206^-$ M1 macrophages, **(C)** $CD137^+$ $CD8^+$ cytotoxic T cells, **(D)** $CD137^+$ $CD4^+$ T cells, **(E)** $CD20^+$ B cells.

baseline is significantly associated with favorable outcomes with ipilimumab therapy in patients with melanoma (30).

Exploratory biomarker studies (4–7) using pivotal trials, including CheckMate-214 (1) and CheckMate-025 (31, 32), have been conducted to predict ICI treatment responses. In the CheckMate-214 trial, PD-L1 IHC, whole exome sequencing and RNA sequencing were performed to evaluate PD-L1 positivity, tumor mutation burden, indel burden, human leucine antigen class I zygosity, the PBRM1 mutation status, and gene signature scores (4). Although the tumor mutation burden and genomic instability can serve as robust predictors of an ICI response in various cancers, these expected factors, as well as PD-L1 positivity, were not found previously to be associated with the clinical benefits of a nivolumab plus ipilimumab combination (4). Besides the PD-1/PD-L1 axis and CTLA-4 for these checkpoint inhibitors, there are several other checkpoints such as PD-L2, T cell immunoglobulin and mucin domain containing 3 (TIM3), and lymphocyte activating 3 (LAG3), which may be associated with immune response (33–35). In the CheckMate-025, -010, and -009 trials, the tumor mutation burden and CD8+ T cell infiltration level were not predictive of second-line nivolumab monotherapy in patients previously treated with tyrosine kinase inhibitor (5–7). However, these predictive values may vary depend on treatment settings and types of ICIs. In this study, the combination of nivolumab with ipilimumab was administered as first-line, and different from nivolumab monotherapy, limited the determination of its predictive values. Unlike previous studies, we here directly examined various immune cells in RCC tissue samples that are the major players in the TME associated with antitumor activity. Moreover, our mIHC approach enhanced the quality of the TME analysis, considering that the difference between certain T cell subsets is not detectable by conventional IHC.

It has been proposed that with the investigation of specific TME components and their recognized impact on the treatment responses, combination strategies that target distinct immune cell subsets may help overcome treatment resistance (11). Repolarizing macrophages toward an M1 phenotype could promote an immune response and engender synergistic effects with ICIs. Inhibitors of PI3Kγ or mTOR as well as agonists of CD40, TLR4, -7, -8, or -9 can repolarize macrophages towards a proinflammatory phenotype promoting tumor suppression in preclinical studies (36). Considering that the indolamine 2,3 dioxygenase 1 (IDO1) overexpressed by M2 macrophages depletes the essential metabolite tryptophan, which hampers T cell proliferation (37), the combination of epacadostat (IDO1 inhibitor) and pembrolizumab has showed promising results, with an ORR of 47% in 19 patients with advanced RCC previously treated with antiangiogenic agents, irrespective of their risk groups (38). The combination of epacadostat and

TABLE 3 Univariate and multivariate analysis for progression-free survival.

	Progression-free survival			
	Univariate analysis		Multivariate analysis*	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (≥65 years vs. <65 years)	0.30 (0.04-2.23)	0.234		
Sex (male vs. female)	0.23 (0.08-0.69)	0.009		
IMDC (poor vs. intermediate)	2.26 (0.72-7.08)	0.162		
Presence of sarcomatoid component in histology (yes vs. no)	0.78 (0.24-2.54)	0.676		
Previous nephrectomy (yes vs. no)	0.75 (0.23-2.48)	0.642		
$CD8^+$ cytotoxic T cells (high vs. low)	0.74 (0.25-2.22)	0.596		
$CD103^+$ $CD8^+$ tissue-resident T cells (high vs. low)	0.82 (0.27-2.47)	0.726		
$CD137^+$ $CD8^+$ T cells (high vs. low)	0.36 (0.11-1.18)	0.093		
$CD137^+$ $CD4^+$ T cells (high vs. low)	0.41 (0.13-1.34)	0.139		
$FoxP3^-$ $CD4^+$ helper T cells (high vs. low)	0.25 (0.07-0.83)	0.024	0.19 (0.05-0.73)	0.016
$FoxP3^+$ $CD4^+$ regulatory T cells (high vs. low)	0.95 (0.32-2.89)	0.934		
$CD20^+$ B cells (high vs. low)	0.47 (0.15-1.47)	0.194		
$CD68^+$ CD206- M1 macrophages (high vs. low)	0.19 (0.05-0.73)	0.016		
$CD68^+$ $CD206^+$ M2 macrophages (high vs. low)	0.40 (0.12-1.34)	0.136		
$CD11c^+$ MHC class II+ dendritic cells (high vs. low)	1.13 (0.34-3.77)	0.844		
$PD\text{-}L1^+$ cells (high vs. low)	0.57 (0.17-1.89)	0.359		

HR, hazard ratio; CI, confidence interval; IMDC, International Metastatic RCC Database Consortium.
*Multivariate analysis included significant factors identified by univariate analysis (P<0.1).

ipilimumab has also shown a promising ORR of 23% in immunotherapy-naïve melanoma patients (39). The efficacy of the combination of epacadostat with ICIs needs to be further investigated, focusing only on intermediate- or high-risk RCC patients. Moreover, along with the prognostic value of CD137, the efficacy and safety of CD137 agonists alone or in combination with ICIs have been investigated in several studies (40–42). Novel therapeutic strategies targeting the upregulation of CD137 expression or enhancement of CD137 signaling for synergistic effects with ICIs need to be further studied in advanced RCC.

Despite our subgroup analysis with further small samples, significant numbers of immune cells had a trend of higher infiltration in the tumor margin than in the tumor center and stroma. The clinical value of the spatial distribution of immune cells has been reported for other cancer types. The density of Foxp3- CD4+ helper T cells in the tumor margin rather than the tumor center and stroma has previously shown the best capacity for predicting the treatment response in biliary tract cancer patients, and the tumor margin may be the main site of the immune response in these cases (43).

The present study had some limitations of note. First, only a small number of patients treated with nivolumab plus ipilimumab were included. This regimen was of limited use because it is not covered yet by the National Health Insurance Service of Korea when this study was designed. Further, larger-scale studies are needed to confirm the value of significant TME biomarkers. Second, only approximately one in five patients in our cohort died at the time of the analysis and OS data could not therefore be analyzed. Long-term follow-up is necessary because PFS cannot always guarantee a long-term response. Third, TME analysis using mIHC may not represent the entire tissue specimen because it is limited to ROIs. There are particular concerns in this regard when using biopsy specimens rather than surgical specimens. It may be necessary to investigate a wider area of tumor tissues to properly assess any possible clinical applicability of these findings, as well as to validate TME biomarkers associated with an ICI treatment response.

In conclusion, several immune cells in the TME are fully associated with the response to ICIs, particularly Foxp3- CD4+ helper T cells and M1 macrophages. These are new predictive biomarkers and possible future therapeutic targets that could help to further improve survival.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Asan Medical Center

(study number: 2019-1712). The ethics committee waived the requirement of written informed consent for participation.

Author contributions

Study concepts: JL. Study design: JK and JL. Data acquisition: JK, GK, Y-MR, S-YK, H-DK, SY, YC, and JL, Quality control of data and algorithms: JK, GK, Y-MR, and YC. Data analysis and interpretation: JK, GK, and Y-MR. Statistical analysis: JK and GK, Manuscript preparation: JK and GK. Manuscript editing: JK, GK, and JL, Manuscript review: JK, GK, Y-MR, S-YK, H-DK, SY, YC, and JL. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.969569/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Quantification of the infiltration level by certain T cell subsets, $CD20^+$ B cells, and M1 macrophages, according to the spatial distribution in each available dataset.

SUPPLEMENTARY TABLE 1
Implications for each markers of multiplexed immunohistochemistry.

SUPPLEMENTARY TABLE 2
Treatment-related adverse event.

SUPPLEMENTARY TABLE 3
Immune cell infiltration densities between patients with any grade of TRAE and those without any TRAE.

SUPPLEMENTARY TABLE 4
Immune cell infiltration densities between patients with grade 3 hyperglycemia and those without grade ≥ 3 TRAE.

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Immune-based treatment re-challenge in renal cell carcinoma: A systematic review and meta-analysis

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Introduction: The use of immune checkpoint inhibitors (ICIs) as a front-line treatment for metastatic renal cell carcinoma (RCC) has significantly improved patient' outcome. However, little is known about the efficacy or lack thereof of immunotherapy after prior use of anti-PD1/PD-L1 or/and anti-CTLA monoclonal antibodies.

Methods: Electronic databases, including PubMed, EMBASE, Medline, Web of Science, and Cochrane Library, were comprehensively searched from inception to July 2022. Objective response rates (ORR), progression-free survival (PFS), and ≥ grade 3 adverse events (AEs) were assessed in the meta-analysis, along with corresponding 95% confidence intervals (CIs) and publication bias.

Results: Ten studies which contained a total of 500 patients were included. The pooled ORR was 19% (95% CI: 10, 31), and PFS was 5.6 months (95% CI: 4.1, 7.8). There were ≥ grade 3 AEs noted in 25% of patients (95% CI: 14, 37).

Conclusion: This meta-analysis on different second-line ICI-containing therapies in ICI-pretreated mRCC patients supports a modest efficacy and tolerable toxicity.

KEYWORDS

immunotherapy, immune checkpoint inhibitor, rechallenge, salvage, second-line, VEGF TKI, renal cell carcinoma

Introduction

Renal cell carcinoma (RCC) is a commonly diagnosed urological malignancy with rising incidence rates (1). Despite decreasing mortality rates in developed countries, advanced RCC remains lethal and thus further progress in the current therapeutic armamentarium and sequencing of systemic therapies is needed. Clear-cell RCC comprises 75% of RCC cases (2, 3).

Until recently, standard first-line treatment therapies for metastatic clear cell renal cancer (mRCC) have been mostly targeted against signaling through the vascular endothelial growth factor receptor (VEGFR), either *via* use of tyrosine kinase inhibitors (TKIs) such as sunitinib and pazopanib (4, 5), or monoclonal antibodies i.e. bevazicumab (6). Patients with disease progression after treatment with first-line anti-angiogenic agents (AA), were destined to receive another VEGFR TKI or/and mTOR inhibitor (7).

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment landscape of RCC, initially at second-line with superiority of nivolumab over everolimus in the CheckMate 025 study (8) and most recently in the first-line setting with ICI-ICI and ICI-VEGFR TKI combinations (9–12). ICIs approved in advanced RCC are monoclonal antibodies against immune checkpoints including the programmed cell death protein 1 (PD-1) or its ligand (PD-L1) and CTLA-4 (13). The binding of cancer cells to immune cells through these checkpoints leads to immune response downregulation and subsequent cytokine release inhibition which, in turn reduces the cytotoxic T-cell activity against tumors (13). This process is reversed by ICIs.

The expanded use of immunotherapy and VEGFR TKIs (ICI-ICI and ICI-VEGFR TKI combinations) in the front-line setting is changing the landscape of subsequent therapies as well. As a result, choosing between available beyond first-line options upon progression has become more challenging. In this context, it remains elusive whether a re-challenging approach, particularly with respect to ICIs could lead to clinically meaningful responses in later lines of therapy in patients with metastatic RCC. In this systematic review and meta-analysis, we provide insight to the efficacy and safety of immunotherapy as a second-line treatment in patients with mRCC who were previously treated with ICIs.

Materials and methods

Eligibility criteria

This study developed the inclusion and exclusion criteria based on "PICOS" principles. Inclusion criteria were as follows: (i) Design of studies, prospective, retrospective or ambispective; (ii) patients (P), patients with metastatic RCC who received at least one prior line of systemic therapy that included an immune checkpoint inhibitor; (iii) intervention (I), second-line immune checkpoint inhibitor; (iv) control (C), not-applicable; (v) outcomes (O), the primary endpoints were objective response rate (ORR), which was defined as percentage of complete (tumor disappearance), or partial (tumor shrinkage ≥ 30%) decrease in the baseline sum of the longest diameter of target lesions and progression-free survival (PFS), which was defined as the length of time that patients lived with the tumor without evidence of progression. The secondary endpoint was ≥ grade 3 toxicity, which according to the Common Terminology Criteria for Adverse Events (CTCAE) was defined as severe or medically significant but not immediately life-threatening adverse events or resulting in hospitalization or prolongation of hospitalization indicated, disabling or limiting self-care activities of daily living (ADL).

Search methodology

The selection and systematic review of clinical studies were performed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (14). The search was limited to studies published in English. We searched PubMed, the Cochrane Library, EMBASE and Web of Science electronic database. Eligible studies were obtained, using search terms (i) renal OR kidney; (ii) cancer OR carcinoma OR tumor OR neoplasm; (iii) renal OR kidney AND cancer OR carcinoma OR tumor OR neoplasm; (iv) metastases OR metastatic; (v) renal OR kidney AND cancer OR carcinoma OR tumor OR neoplasm AND metastases OR metastatic; (vi) salvage OR second-line; (vi) immunotherapy OR immune checkpoint inhibitor; (vii) renal OR kidney AND cancer OR carcinoma OR tumor OR neoplasm AND metastases OR metastatic AND salvage OR second-line AND immunotherapy OR immune checkpoint inhibitor. We included studies up until July 2022. A manual screen of study references was also conducted to obtain possibly relevant literature. After excluding repeated studies, we screened all articles based on their title, abstract, and full text.

Data extraction

Using a standardized data extraction form, two investigators independently extracted the following data from each study: (i) Study ID, including the name of the first author and publication year; (ii) country where the study was performed; (iii) study subjects, number of participants and their ages; (iv) treatment regimens; and (v) treatment outcomes, including objective response rate (ORR), progression-free survival (PFS), and ≥ Grade 3 toxicity. For reports of the same study at different

follow-up periods, data from the last report were used for analysis.

Statistical analysis

Based on the data available from the studies we analyzed the Objective Response Rate (ORR), the median progression-free survival (PFS) and the proportion of patients with ≥3 Grade AEs and. ORR and the proportion of patients with Grade ≥3 AEs needed methods suitable for rates and proportions. We used the statistical software Stata, with the Freeman-Tukey double arcsine transformation implemented in metaprop (15) and metan and the logit transformation (16) implemented with metan. For PFS we used its logarithm along with 95% confidence intervals provided by the studies (17). In all cases we used the inverse-variance random-effects method of DerSimonian and Laird (18) in order to account for between studies variability (heterogeneity). The I-squared index was used to quantify heterogeneity. Publication bias was estimated using the Egger regression test (19) and the Begg's and Mazumbar's rank correlation test (20).

Results

Study selection outcome

Among the publications retrieved using electronic search (N=89), 10 studies were eligible for the present meta-analysis, including a total of 500 patients (21–30). The detailed flowchart of the selection process for eligible studies is depicted in Figure 1.

Study characteristics

The studies included in this meta-analysis were published between 2020 and 2022. With regards to treatment, 7 studies used nivolumab plus ipilimumab, or nivolumab alone as second-line therapy (21, 23–28), one study used the combination of pembrolizumab and lenvatinib (29) and another used the combination of atezolizumab and bevacizumab (30). A multicenter retrospective cohort study analyzed various combinations including nivolumab/ipilimumab, pembrolizumab/axitinib, pembrolizumab/bevacizumab, atezolizumab/investigational agent, nivolumab/investigational agent, avelumab/chemotherapy, spartazilumab/investigational agent, and monotherapies with pembrolizumab, nivolumab, or durvalumab (22). All studies reported ORR and PFS as outcomes as well as safety data. The clinical characteristics of the included studies are presented in Table 1.

ORR

The pooled ORR using the Freeman-Tukey double arcsine transformation was calculated equal to 0.19 (95% CI: 0.10, 0.31), with I-squared equal to 88.30%. Similar estimates were obtained with the logit transformation, ORR=0.19 (95% CI: 0.11, 0.31) with I-squared=85.9% (Figure 2).

PFS

The pooled PFS was found equal to 5.655 months (95% CI: 4.120, 7.762 months) with I-squared equal to 76.9% (Figure 3).

Serious AEs

The pooled proportion of patients with Grade ≥3 AEs using the Freeman-Tukey double arcsine transformation was calculated equal to 0.25 (95% CI: 0.14, 0.37), with I-squared equal to 88.79%. Similar estimates were obtained with the logit transformation, ORR=0.25 (95% CI: 0.16, 0.37) with I-squared=86% (Figure 4).

Publications bias

For pooled ORR analysis, both tests for publication bias, including Egger's and Begg's, suggested the presence of it (p-value<0.0001 and 0.012 respectively) (Figure 5). With respect to pooled PFS, neither test suggested any evidence of it (p-value=0.078 and 0.283 respectively) (Figure 6). Regarding the pooled proportion of patients with Grade ≥3 toxicity, both tests for publication bias suggested the presence of it (p-value=0.016 and 0.048 respectively) (Figure 7).

Discussion

This systematic review and meta-analysis were conducted to answer the question of whether ICI rechallenging in patients with mRCC who have progressed after anti-PD-1/PD-L1 as part of front-line therapy is a safe approach that could result in clinically meaningful responses. Our study showed an ORR of 19% for beyond first-line ICI treatment combinations, mostly including nivolumab/ipilimumab, lenvatinib/pembrolizumab, atezolizumab/bevacizumab and to a lesser extent other ICI/VEGFR TKI combinations. Pooled PFS was 5.655 months and grade ≥3 adverse events were experienced by one quarter of patients (25%).

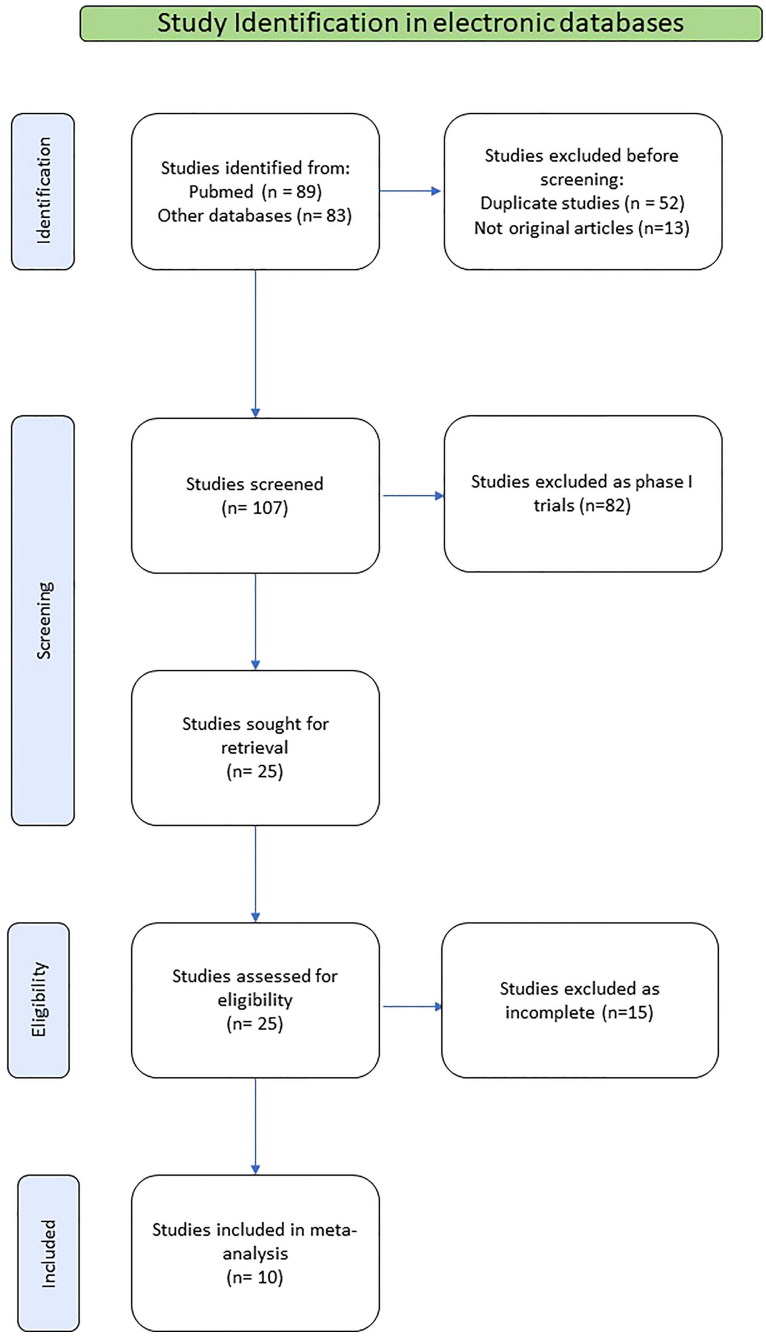

FIGURE 1
Flowchart of the selection process for eligible studies.

The synthesis of this meta-analysis involved a heterogenous group of phase II prospective trials with adaptive or fixed design, retrospective studies and a control study with varying sample sizes, first-line treatments and number of previous lines. Among the included studies, three phase II non randomized trials evaluated salvage therapy with nivolumab plus ipilimumab in patients who had received nivolumab monotherapy as first-line treatment (HCRNGU16-260, TITAN-RCC, OMNIVORE) (24–26) and were non-responders. The lowest ORR was observed in the OMNIVORE trial (4%), which might be attributed to patients receiving only 1-2 cycles of combination second-line therapy, whereas, the other two trials administered 2-4 cycles.

TABLE 1 Characteristics of included studies.

Study (author,year)	Design	N	Age (medianl	IMDC(%) fav/ int/poor	Prior line	2nd line	N of cycles	ORR (%)	PFS (mos)	Grade 3 M.s (%)
Gul et a l. 2020 (21)	retros pective	45	62	20/64/7	ICI± other	Nivo +lpi	≥1	9/45 (20%)	4(0.8-19)	6/45(13%)
Ravi et al. 2020 (22)	retrospective	69	61	19/65/12	ICI±ICI or ICI+AA	ICI±ICI or ICI+AA	2-8	15/64 (23%)	5.7(3.2-7.6)	11/69 (16%)
Choueiri et al. 2020 FRAClION-RCC) (23)	Phase II	46	NA	NA	ICI AA (80%)	Nivo +lpi	≥1	7/46 (15.2%)	4 (2.3-7.9)	13/46(28.3%)
McK ay et al. 2020 (OMNIVOR E) (24)	Phase II	57	63	34.1/5 6.8 /9.	Nivo	Nivo +lpi	1-2	2/57 (4%)	4.7 (2.7-8.3)	14/57 (25%)
Grimm et a l. 2021(TITAN-RCC) (25)	Phase II	28	65	0/71/25	Nivo	Nivo +lpi	24	3/28 (11%)	3.7 (24.5)	NA
Atkins et al. 2022 (HCRN GU16-260) (26)	Phase II	35	65	17.2/77.1/5.7	Nivo	Nivo +lpi => Nivo	4	4/3 5 (11.4%)	8.3(5.5-10.9	15/35 (42.9%)
Yang et al. 2021(27 I	retrospective	27	61.4	6/13/ 5	Nivo	Nivo +lpi => Nivo	≥1	5/22 (23%)	4(2.4-6.2)	5/27(18.5)
Vauchier et al. 2022 (28)	ambispective	45	59	23/25/53	ICI±ICI, ICI+AA	Nivo ±lpi	≥1	7/45 (16%)	3.5 (2.8-9.7)	2/45 (4%)
Lee et al. 2021(KEYNOTE-146) (29)	Phase lb/11	104	60	17/59/24	ICI±ICI, ICI+AA	Pembro+Lenva	8	58/104 (55.8%)	12.2 (9.5-17.7)	59/104(57%)
Powles et a l. 2021 (1Mmotion150) (30)	Phase II	44	61	NA	Atezo	Atezo +Beva	≥1	11/44 (25%)	8.7(5.6-13.7)	NA (for the entire study N=103, 31/103 (30%)

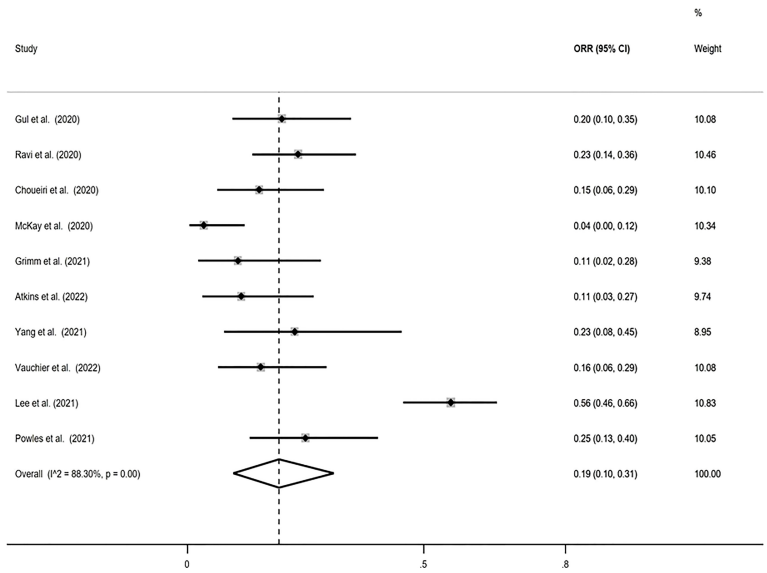

FIGURE 2
Forest plot displaying the pooled objective response rate (ORR) proportion in random-effects meta-analysis with the Freeman-Tukey double arcsine transformation.

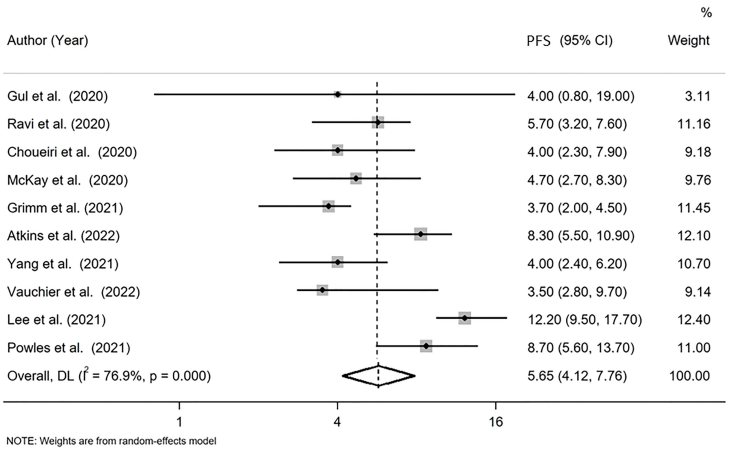

FIGURE 3
Forest plot displaying the pooled median progression-free survival (PFS) in random-effects meta-analysis.

Overall, ICI monotherapy followed by salvage ICI combination did not achieve good responses neither in the first- nor in the second-line settings.

In the study of Ravi et al. (22) which included various ICI/ICI and ICI/VEGFR TKI combinations, higher ORR at second-line was observed in patients who responded in first-line, compared to those who progressed or had stable disease in the first-line, but remained similar to those receiving first-line monotherapy, suggesting that responses can be observed in second-line immunotherapy and that resistance can be overcome when using different ICIs combined with VEGFR TKIs (22). Similar and higher ORRs were noted in the

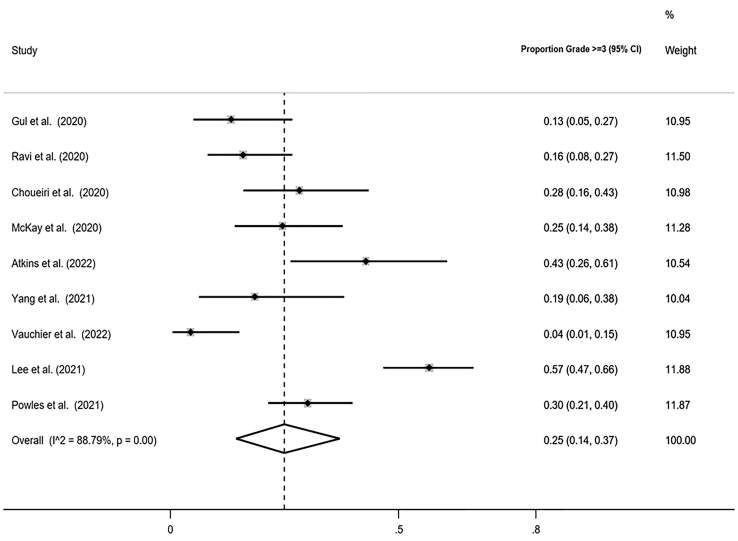

FIGURE 4
Forest plot displaying the pooled Grade ≥3 proportion in random-effects meta-analysis with the Freeman-Tukey double arcsine transformation.

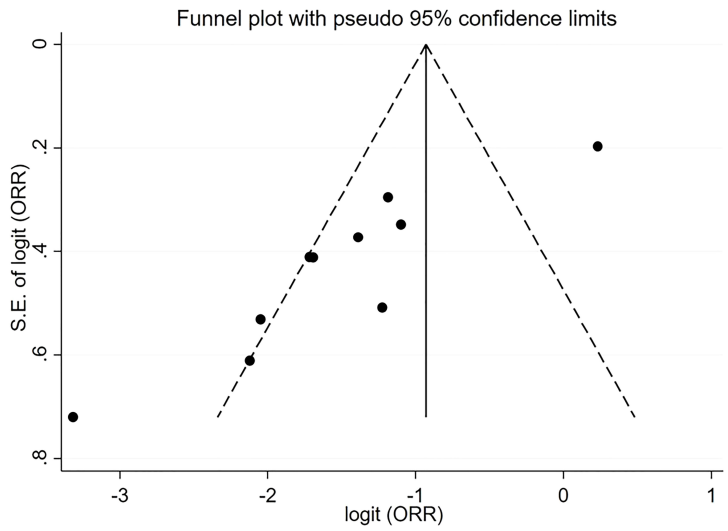

FIGURE 5
Funnel plot with pseudo 95% confidence limits for the estimation of the publication bias for the objective response rate (ORR) proportion with the logit transformation.

other two trials that tested an ICI/VEGFR TKI combination beyond first-line (29, 30). In the pembrolizumab-lenvatinib study of Lee et al. (29), more than half (56%) of patients responded despite the fact that two-thirds (65%) of patients had already received a TKI as part of first-line combination therapy, while in the atezolizumab-bevacizumab study of Powles et al. (30) a quarter (25%) of all VEGFR inhibition-naïve patients responded This could imply a sensitizing effect of VEFGR pathway inhibition to further ICI or/and an immune-independent way of completely avoiding cross-resistance particularly in VEGFR TKI-naïve patients. Another important observation across different studies is that the poorest responders to beyond first-line combinations included those with a high burden of metastases (≥1-3), presence of brain metastatic sites and deteriorated ECOG performance status (≥ 2) (21, 28). In this patient population, the ICI/VEGFR TKI

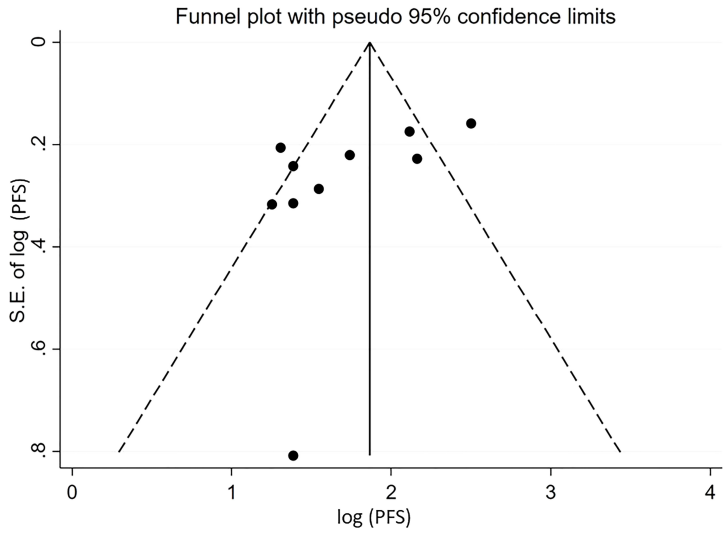

FIGURE 6
Funnel plot with pseudo 95% confidence limits for the estimation of the publication bias for the median progression-free survival (PFS) with the logarithm transformation.

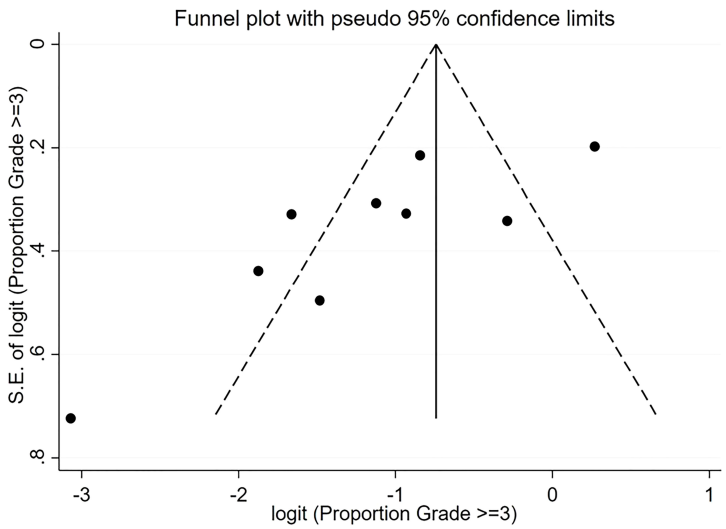

FIGURE 7
Funnel plot with pseudo 95% confidence limits for the estimation of the publication bias for the Grade ≥3 proportion with the logit transformation.

combination seemed to be more active if indirectly compared to double ICI, judging from the high ORR (55.8%) and prolonged PFS (12.5 months) of the pembrolizumab/lenvatinib regimen (29).

The results of this meta-analysis are in line with two previous meta-analyses that examined the activity of salvage nivolumab/ipilimumab after prior PD-1 blockade with nivolumab (31, 32). They reported a pooled ORR of 10% (31) and 14% (32), respectively, while PFS ranged between 3.7 and 5.5 months (32). Our study further complements these two meta-analyses by additionally providing a more comprehensive landscape of how ICI works beyond first-line overall, either as ICI doublet or as ICI/VEGFR TKI combination, particularly having also included the studies of Lee et al. (29) and Powles et al. (30), as well as updated data from previous nivolumab/ipilimumab studies.

There were no new safety signals, and all three meta-analyses, including ours reported a comparable percentage of pool incidences of ≥ grade 3 events of 25%-26% (27, 31).

Two additional retrospective studies focusing solely on ICI/TKI combinations reported relatively high objective response (51% and 37.5% respectively) and median PFS (11.6 and 14.2 months in the second-line setting (32, 33). These two studies were excluded from our meta-analysis due to high inherent heterogeneity with respect to including a heavily pre-treated population with at least 2 prior lines of therapy (32) less than half of whom had received ICI during first-line therapy (32, 33).

Because this meta-analysis aimed to explore as many ICI-inclusive options as possible in beyond first-line treatment of mRCC, variations in the types and duration of administration of treatment regimens used, inconsistent timing between anti-PD-1/PD-L1 failure and salvage ICI-containing second-line therapy among these studies, inconsistent baseline clinical data, including IMDC and MSKCC prognostic groups, were inevitable and may have all resulted in the heterogeneity observed. Another limitation of this analysis is derived from the inherent sparseness of ICI-rechallenge studies in mRCC. A greater number of prospective clinical trials with more homogenous inclusion criteria, treatment design and longer follow up would help minimize heterogeneity among studies, and provide a clearer picture on these patients' outcomes. Individual data could also provide a clearer image on the putative correlation between first and subsequent lines of treatment with ICIs for eligible patients. All data was retrieved directly from publications. Additionally, one of the studies was only available in abstract form; however, it was included due to its unique design. This fact, along with a publication bias calculated in the logit scale, indicate that results should be interpreted with caution.

This meta-analysis on different second-line ICI combinations in ICI-pretreated mRCC patients supports a modest efficacy and tolerable toxicity. A careful selection of the subset of ICI-pretreated patients who are most likely to benefit from ICI-containing therapies beyond first-line should take place for treatment decision-making. Phase III randomized trials of various ICI-TKI combinations after prior ICI are currently ongoing (NCT04987203, NCT04338269, NCT03793166). For example, atezolizumab combined with cabozantinib is currently being tested in the pivotal, global phase III CONTACT-03 trial in patients with inoperable, locally advanced or metastatic renal cell carcinoma (RCC) who progressed during or following treatment with an ICI (NCT04338269). The concept of ICI rechallenge after progression is expanding in other primaries. Although

randomized comparisons are lacking, preliminary evidence from individual cases (34, 35) and metaanalyses (36, 37) support its safety with low to modest efficacy, e.g. 8-13% ORR in non-small cell lung cancer, depending on the clinical context.

Author contributions

PV, VT, and PB conceptualized and designed the study. PV, MP, and IT performed the search and drafted the manuscript. MP and PV performed the data extraction. KE-K, PK, and PB analyzed the data. IT, KE-K, PK, and PB provided the clinical imaging data of the patients. MP, LM, MS, PB, VT, and PV reviewed and revised the original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The value of the multidisciplinary team in metastatic renal cell carcinoma: Paving the way for precision medicine in toxicities management

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The new landscape of treatments for metastatic clear cell renal carcinoma (mRCC) is constantly expanding, but it is associated with the emergence of novel toxicities, adding to up to those observed in the tyrosine-kinase inhibitor (TKI) era. Indeed, the introduction of immune checkpoint inhibitors (ICIs) alone or in combination has been associated with the development of immune-related adverse events (irAEs) involving multiple-organ systems which, even if rarely, had led to fatal outcomes. Moreover, due to the relatively recent addition of ICIs to the previously available treatments, the potential additive adverse effects of these combinations are still unknown. A prompt recognition and management of these toxicities currently represents a fundamental issue in oncology, since it correlates with the outcome of cancer patients. Even if clinical guidelines provide indications for the management of irAEs, no specific protocol to evaluate the individual risk of developing an adverse event during therapy is currently available. A multidisciplinary approach addressing

appropriate interventions aimed at reducing the risk of any insidious, severe, and/or dose-limiting toxicity might represent the most efficacious strategy to timely prevent and manage severe irAEs, allowing indirectly to improve both patients' cancer-specific survival and quality of life. In this review, we reported a five-case series of toxicity events that occurred at our center during treatment for mRCC followed by the remarks of physicians from different specialties, pinpointing the relevant role of an integrated and extended multidisciplinary team in a modern model of mRCC patient management.

KEYWORDS

multidisciplinary team (MDT), metastatic renal cell carcinoma (mRCC), endocrinological toxicity, cardiovascular toxicity, liver toxicity, nephrological toxicity, cutaneous toxicity

1 Introduction

Renal cell carcinoma (RCC) is an insidious neoplasm, accounting for approximately 2% of global cancer diagnoses and deaths, whose incidence will further increase worldwide. Cancers of the kidney and renal pelvis have rapidly become more common in the developed world over the past decades (1). According to 2018 GLOBOCAN data, an estimated 403,000 people per year are diagnosed with kidney neoplasms, constituting 2.2% of all cancer diagnoses (2). In Italy, AIOM estimates that for the year 2020, the number of new cases of kidney cancer is 13,500 and deaths 4,900, accounting for 2.4% of all cancer-related deaths (3). The overall survival (OS) of patients affected by RCC has improved year after year: compared with the 90s and 2000s, an increase in OS has been shown, respectively, of 25% and 11%, both in USA and Italy, representing one of the best results obtained during the last 10 years (4). Indeed, with the arrival of new innovative molecules, such as immune checkpoint inhibitors (ICIs) and novel tyrosine kinase inhibitors (TKIs), the prognosis of RCC in advanced stages has been profoundly improved. According to European guidelines (5), the first-line treatment of metastatic RCC (mRCC) depends on the IMDC (International Metastatic RCC Database Consortium) risk group, defined by six negative clinical prognostic factors that stratify patients with mRCC in three subgroups: good, intermediate, and poor-risk. Accordingly, patients without negative factors have a good prognosis and may obtain a longer survival; patients with one or two factors are at an intermediate risk of death, with a median OS of about 23 months; patients with three or more factors are expected to have a poor outcome, with a median survival of about 8 months (6). The first-line therapy in the favorable-risk mRCC should be a TKI in combination or not with an ICI (in Italy, the current approved combination is axitinib plus pembrolizumab according to the KEYNOTE-426 trial (7); in the intermediate or poor risk, other than a TKI+ICI combination, dual immuno (IO) combination (IO–IO) with ipilimumab and nivolumab can also be used, according to the CheckMate 214 trial (8). Other combinations like cabozantinib and nivolumab, and lenvatinib plus pembrolizumab, as reported in the CheckMate 9ER (9) and CLEAR (10) studies, respectively, were recently approved by EMA for any IMDC risk class mRCC, but they are still not approved in Italy by AIFA; thus, we will not further discuss their use.

In KEYNOTE 426, the most common related grade 3 or higher adverse effects described are (≥10% patients in either group) as follows: hypertension [95 (22%) of 429 patients in the pembrolizumab plus axitinib group vs. 84 (20%) of 425 patients in the sunitinib group), alanine aminotransferase increase [54 (13%) vs. 11 (3%)], and diarrhea [46 (11%) vs. 23 (5%)]; deaths from adverse events (AEs) occurred in 19 (4%) of 429 patients in the pembrolizumab plus axitinib group (acute coronary syndrome, acute myocardial infarction, cardiac failure, cardiac tamponade, myocarditis, unknown cause, general physical health deterioration, sudden cardiac death, necrotizing fasciitis, pneumonia, plasma cell myeloma, myasthenia gravis, pleural effusion, pneumonitis, pulmonary embolism, pulmonary thrombosis, and respiratory failure, in one patient each; and cardiac arrest in two patients) (6). In CheckMate 214, the most common adverse reactions (≥20%) of any grade reported in patients treated with nivolumab plus ipilimumab (n = 547) were fatigue (58%), rash (39%), diarrhea (38%), musculoskeletal pain (37%), pruritus (33%), nausea (30%), cough (28%), pyrexia (25%), arthralgia (23%), decreased appetite (21%), dyspnea (20%), and vomiting (20%). The most frequent serious adverse reactions reported in ≥2% of patients were diarrhea, pyrexia, pneumonia, pneumonitis, hypophysitis, acute kidney injury, dyspnea, adrenal insufficiency, and colitis. Severe or fatal cases have also been reported with adverse reactions involving different organs and systems, especially cardiovascular (myocarditis, pericarditis, vasculitis), gastrointestinal (pancreatitis to include increases in serum amylase and lipase levels, gastritis, duodenitis), musculoskeletal and connective tissue (myositis/polymyositis,

rhabdomyolysis, and associated sequelae including renal failure, arthritis, polymyalgia rheumatica), and endocrinological (hypoparathyroidism) diseases (8).

In case of disease progression, the most frequently used second-line treatment is the multi-tyrosine kinase inhibitor cabozantinib. However, a well-defined treatment algorithm has not yet been established (11). During cabozantinib treatment, most adverse reactions occur early in the course of treatment and include hypocalcemia, hypokalemia, thrombocytopenia, hypertension, palmar–plantar erythrodysesthesia syndrome (PPES), proteinuria, and gastrointestinal (GI) events (abdominal pain, mucosal inflammation, constipation, diarrhea, vomiting). In the METEOR trial, patients pretreated with vascular endothelial growth factor (VEGF)-targeted therapy reported dose reductions and dose interruptions due to an AE in 59.8% and 70%, respectively. Finally, when cabozantinib was given in combination with nivolumab in first-line advanced renal cell carcinoma, according to the most recent trial, CheckMate 9ER, dose reduction and dose interruption of cabozantinib due to an AE occurred in 54.1% and 73.4% of patients. The rates of treatment-related adverse events of grade 3 or higher were 60.6% (6.9% diarrhea, 7.5% PPES, 12.5% hypertension, 5.3% increased ALT level, 9.4% hyponatremia, 5.9% hypophosphatemia) in the nivolumab-plus-cabozantinib group and 50.9% (4.4% diarrhea, 7.5% PPES, 13.1% hypertension, 4.7% decreased platelet count and 3.8% neutropenia/anemia) in the sunitinib group.

New targeted agents as well as a new combo with immunological drugs expand treatment chances for mRCC patients but are associated with more novel toxicities as compared with those observed with the previously available medications, such as sunitinib or pazopanib. Moreover, due to the relatively recent introduction of these combinations in clinical practice, their cumulative dose adverse effects are still unknown. However, the most frequently occurring affect the skin, colon, endocrine organs, liver, and lungs. Others are very infrequent but may be very serious, even lethal, such as neurological disorders and myocarditis (12).

A prompt recognition and management of these toxicities represents a fundamental issue in oncological clinical practice, since it correlates with the outcome of cancer patients. In this context, it is therefore essential to prevent any adverse events that may lead to a discontinuation of treatment or a dose reduction. A multidisciplinary management of the various toxicities that may arise during treatment of patients with mRCC will obviously help patients to achieve better treatment compliance (10). Indeed, multidisciplinary teams (MDTs) have been recommended to improve cancer care and outcomes for all managed patients (13). Patients should be investigated for preexisting risk factors to contain the effect of those that are modifiable, even if consensus recommendations for the identification of a population most at risk of toxic events are currently lacking. For those patients with baseline organ impairments, a multidisciplinary approach should be strongly recommended for an early identification of potential adverse events. The limited knowledge of the pathophysiology and management of life-threatening complications relating to new cancer drugs presents a need to provide a more heterogenous staff, with oncologists, and organ specialists with evidence-based algorithms and requires a multidisciplinary approach (14).

Nowadays, there is no specific protocol to evaluate the risk of developing an adverse event from the novel therapies for mRCC patients. Therefore, we reported a case series and literature review, describing five examples of critical toxicities that occurred in our center during treatment for mRCC and how they should be managed, with the aim to highlight the role of MDT in the genitourinary cancer unit for an integrative management of mRCC patients.

2 Patients and method

This study reported a case series of mRCC treated at Sapienza University Oncological Units with a special focus on the different toxicities that occurred during IO-based or targeted therapies for mRCC. Clinical records of five patients affected with clear cell renal carcinoma, treated in metastatic setting, and discussed in our multidisciplinary team for drug-related toxicity were analyzed for the present study. The first case reported a multidisciplinary management of endocrinological toxicity during the IO combo with nivolumab (3 mg per kilogram of body weight) plus ipilimumab (1 mg per kilogram) intravenously every 3 weeks for four doses, followed by nivolumab (3 mg per kilogram) every 2 weeks. The second case involved a patient, treated before with pembrolizumab plus axitinib at a standard schedule (pe 200 mg plus axi 5 mg twice a day, administered at a 3-week interval) followed in second line with cabozantinib, who reported nephrological toxicities. The third and fourth cases entailed patients treated in the first-line treatment with standard pembrolizumab plus axitinib, during which they showed liver and cardiological toxicities, respectively. Finally, the fifth case was about a multidisciplinary management of dermatological toxicity due to cabozantinib. The severity of adverse events was graded according to CTCAE version 4.0. At the time of first oncological visit, all our patients signed informed consent in which the consent to the use of their data for research purposes is included.

3 Results

3.1 Case 1: Multidisciplinary management of endocrinological toxicities

3.1.1 Case presentation

A 69-year-old man underwent right nephrectomy surgery in May 2019 for a renal carcinoma with sarcomatoid (Ki67 40%,

p53 <1%) and poorly differentiated clear renal cell components, pT3a pNx, stage III according to AJCC 2017. The postsurgery total-body contrast-enhanced computed tomography (CT) showed suspected pulmonary and mediastinal lymph node metastasis, confirmed by transbronchial needle aspiration (TBNA). According to the prognostic criteria of Motzer and Coll and Heng (15, 16), for the presence of hypercalcemia and the time to start systemic treatment less than 1 year after diagnosis, the patient belonged to the intermediate prognosis group. In August 2019, he began immunotherapy with nivolumab (3 mg per kilogram of body weight) plus ipilimumab (1 mg per kilogram) intravenously every 3 weeks for four doses, followed by nivolumab (3 mg per kilogram) every 2 weeks. In view of the combination of an anti-CTLA4 and an anti-PD1, a periodic monitoring of thyroid function (TSH, FT3, FT4), for each of the first four doses, and hypophyseal function (basal ACTH and cortisol) was performed (17). At the third administration, we observed a grade 1 (G1) hyperthyroidism according to Common Terminology Criteria for Adverse Events 5.0 (CTCAE) [↓TSH 0.03 μIU/ml (normal range 0.27–4.2), ↑FT4 2.29 ng/dl (normal range 0.7–1.48), FT3 2.5 pg/ml (normal range 1.71–3.71)], without related symptoms, and as recommended by guidelines, immunotherapy was continued with laboratory monitoring. At the fourth cycle, the G1 hyperthyroidism was stable. The revaluation CT showed a partial response and nivolumab was continued. At the first maintenance cycle, the patient was asthenic, with muscle weakness, constipation, and limitation of daily activities. Laboratory tests showed normal pituitary function and confirmed G2 hypothyroidism [TSH 130.0 μIU/ml (0.27–4), ↓ FT4 0.10 ng/dl (0.7–1.48), FT3 2.0 pg/ml (1.71–3.71) ↑ thyroglobulin 187 ng/ml (normal range 3–40)]. Treatment was discontinued until control of symptoms, from December 2019 to February 2020, and a different thyroid hormone supplementation with levothyroxine was prescribed (Figure 1). In February 2020, the patient started therapy with nivolumab, reaching in August 2020 an optimal response with a resolution of hypothyroidism at the end of October 2020.

In December 2020, the patient had G2 asthenia, restriction of activities of daily living but not of personal care, dizziness, headache, non-alterations of vision, and G1 diarrhea. Laboratory tests showed hypoglycemia (72 mg/dl), hyponatremia, reduced levels of ACTH (5.4 pg/ml; normal range 7.2–63.3 pg/ml), and cortisol (3.5 μg/l; normal range 23–194 μg/l) at 8:00 a.m., TSH (0.18 μUI/ml), and FT4 (0.6 ng/dl). The ACTH stimulation test (1 μg) showed an insufficient adrenal response (basal cortisol: 3.3 μg/dl; cortisol 60 min: 6.8 ug/dl). Due to the headache and dizziness, a magnetic resonance imaging (MRI) of the brain was performed and highlighted the radiological signs of a meningeal irritation attributable to an hypophysitis (Figure 2).

Then, in the presence of secondary adrenal insufficiency and secondary hypothyroidism, a diagnosis of immuno-related hypophysitis was placed, also supported by radiological imaging. The ICI was stopped, and an adrenal and thyroid replacement therapy (levothyroxine, 125 μg in the morning and cortisone acetate 25 mg upon awakening and 12.5 mg in the early afternoon) was administered. The immunotherapy was suspended for a month and resumed after normalization of pituitary function. The patient still maintains a complete radiological response with an OS of 29 months.

3.1.2 Endocrinologist opinion

The incidence of thyroid disorders in course of immunotherapy is rarely higher than G2, due to the frequent monitoring of thyroid function that allows to an early detection. In particular, a meta-analysis of 28 studies, which included more than 7,500 patients, showed an incidence of hyperthyroidism

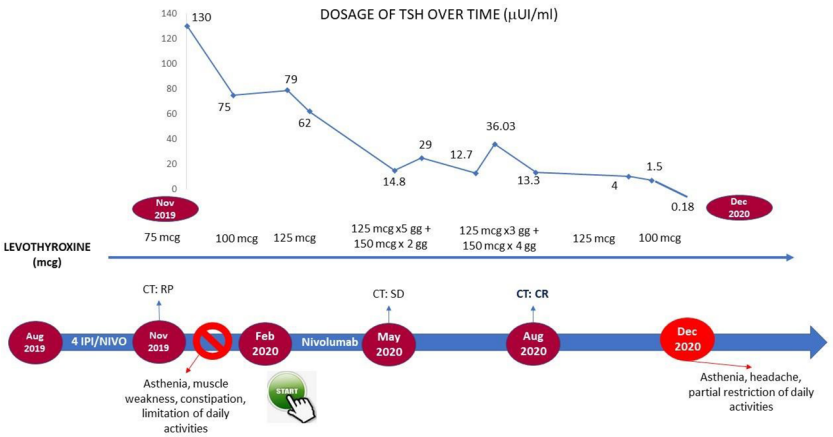

FIGURE 1
Timeline management of cutaneous toxicity in the course of cabozantinib treatment.

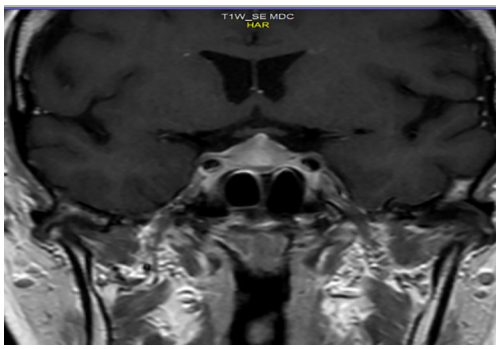

FIGURE 2
MRI: Radiological signs of hypophysitis. Inhomogeneous and enlarged appearance of the pituitary gland showing "tent" morphology due to tension of the meninges with thickening of the pituitary stalk.

and hypothyroidism, under combined anti-CTLA4/anti-PD1 treatment, of 8% and 13.2% versus 3.2% and 3.9% in the course of an anti-PD1 treatment, respectively (8). Thyroid disorders are more frequently primary, rarely secondary to pituitary gland dysfunction. Both the hyper- and hypothyroidism are different manifestations of the same pathological entity: a dextrose thyroiditis mediated by cytotoxic T lymphocytes against the thyroid gland (9, 10). Nowadays, international guidelines do not provide a clear direction regarding the management of G2 hypothyroidism. In fact, according to the AIOM Italian guidelines (11), ICI treatment should be continued, associating it with hormone replacement therapy, whereas the ESMO and ASCO guidelines give the opportunity to stop treatment according to clinical judgment (17, 18). Although many of the studies in the literature are retrospective, in most cases the immunotherapy is continued without further toxicity (19–26). In the clinical case described, on the contrary, the treatment was discontinued for about 3 months.

Pituitary gland disorders are more frequent with anti CTLA-4 than with anti-PD1/PD-L1. The incidence of hypophysitis depends on the dose and drug administered: with ipilimumab, 3 mg/kg is 1%; with ipilimumab, 10 mg/kg is 16%; with nivolumab, 240 mg is 1.1%; and with ipilimumab, 3 mg/kg + nivolumab 240 mg reaches 8% (8, 20). The pituitary damage is apparently caused by monoclonal antibodies and/or activation of T cells directed against antigens shared between cancer cells and pituitary cells or cross-reactive antigens (17, 27, 28). Currently, the guidelines recommend discontinuing treatment and setting up an endocrine replacement therapy, in case of G≥2 immuno-related hypophysitis (17, 18). However, even in this case, in several retrospective studies some patients, despite a G2 toxicity, continued immunotherapy with good control of symptoms (24, 29, 30). It is our opinion to assess the possible interruption on a case-by-case basis, discussing the choice in a multidisciplinary team, as in some circumstances, a good control of symptomatology can be obtained without interrupting the ICI treatment ongoing.

In conclusion, in the present case report, the front-line treatment, still in progress, has been allowed to reach a survival of more than 2 years. An adequate laboratory monitoring is mandatory to manage endocrine toxicities in advance. Of course, a more appropriate diagnostic classification of endocrinological toxicity, together with a more detailed of toxicity degree, is required. In case of G≥2 immuno-related endocrinological disorders, suspension of treatment is not mandatory. A multidisciplinary approach in the management of toxicity is essential to ensuring a correct cost/benefit balance for the patient, favoring therefore greater adherence to treatment while respecting an adequate quality of life. Table 1 shows the biochemical tests that should be performed during treatment with ICIs.

In summary, our case concluded that adequate laboratory monitoring is essential for early intervention in the management of endocrine toxicity. In the presence of endocrinopathy, an accurate diagnosis and a correct definition of the degree of toxicity are needed; if hypothyroidism and adrenal insufficiency occur during treatment with ICIs, it is extremely important to start replacement therapy but discontinuation of immunotherapy is almost never indicated. A multidisciplinary approach to the management of toxicities is essential to ensuring correct continuation of therapy for the patient and also greater adherence to treatment in accordance with an adequate quality of life.

3.2 Case 2: Multidisciplinary management of nephrological toxicities

3.2.1 Case presentation

In June 2008, a 70-year-old man with a history of cerebral ischemia, atrial fibrillation, and hypertension experienced a

TABLE 1 The table summarizes our MDT suggestions and does not reflect any expert consensus or guideline: which exams are recommended by the experts to prevent and identify any adverse event?

Category of toxicity	Which exams are recommended?	When and How?
Endocrinological	ACTH, baseline cortisol, TSH, FT3, FT4	For anti CTLA4 (alone or in combination): every cycle for the first 4 cycles then every 4-6 week
		For anti PD1 or anti PD-L1: every cycle for first 3 months and every second cycle thereafter (cortisol is indicated by symptoms/falling TSH)
		When morning cortisol values are between 3 and 15 ug/dl.
	ACTH test	Peak cortisol levels <18.1 ug/dl at 60 minutes indicates adrenal insufficiency.
Nephrological	Renal function, urine analysis including 24 hours proteinuria and electrolytes	Baseline and every cycle
Liver	Hepatitis baseline screening	Baseline
	Liver function test	At the first occurrence of liver enzyme increase
		Every cycle
Cardiovascular	Blood pressure measurement;	Baseline and weekly in the first 8 weeks
	Comprehensive cardiological evaluation including electrocardiogram, troponine and NT-pro BNP, echocardiogram with strain analysis;	Baseline and in case of symptoms
	Cardiovascular Magnetic Resonance;	In case of symptoms and/or troponine raise and/or ECG change
Dermatological	Clinical examination	Every cycle
	Dermatological evaluation	At baseline in case of patients with history of skin disease At symptoms
	RF dosage and HLA genotype testing	Baseline (only within clinical trial)

persistent abdominal pain and weight loss (5 kg over a year). Imaging revealed a renal lesion of $58 \times 62 \times 55$ mm in the upper pole and pars intermedia of the right kidney, suspected for neoplastic mass, with no other tumor lesions. Therefore, the patient underwent right nephrectomy, and the histological examination revealed a clear-cell type cancer (pT1b pN0, stage I according to TNM/AJCC classification and G2 according to Fuhrman classification). The follow-up was negative until a total body CT scan showed a relapse of disease in the lung, pancreas, and subcutaneous tissue (the histological examination revealed a new metastatic lesion of clear-cell type carcinoma). In January 2021, a first-line therapy for good risk with standard pembrolizumab plus axitinib was administered. Laboratory tests documented a baseline serum creatinine at 1.4 mg/dl. After 5 cycles, in May 2021, for the first time the renal function worsened (serum creatinine 2.1 mg/dl) with a negative urine test. Renal ultrasound did not show any sign of kidney obstruction (e.g., calculi deposits). The patient carried out a nephrological evaluation, and it was decided to replace sartan-based antihypertensive therapy with a calcium antagonist in order to avoid concomitant renal medication damage. Hydration was preserved, and cancer treatment continued. After 2 weeks, creatinine was about 1.5 mg/dl. Oncologic therapy was not stopped. After 2 more cycles of pembrolizumab plus axitinib, acute renal dysfunction was observed again (serum creatinine 2.4 mg/dl). Therefore, according to the ESMO clinical guidelines about the management of immunotherapy-related nephritis (4) and in agreement between oncology and nephrology specialists, therapy with pembrolizumab plus axitinib was withheld, a correct state of hydration was guaranteed, and prednisolone 0.5 mg/kg/die was started. After 2 weeks, serum creatinine was about 1.8 mg/dl and we decided to restart axitinib. After 2 more weeks, serum creatinine was 1.5 mg/dl and combined therapy with pembrolizumab and axitinib was resumed, continuing prednisone 5 mg per day (31).

In September 2021, after nine cycles of therapy, a total body CT showed disease progression: the pancreatic nodule increased in size (from 1.5 × 1 cm to 3.5 × 5.5 cm) and a new lesion appeared in the second liver segment. Thus, we decided for a second-line treatment with cabozantinib 60 mg per day. After 3 cycles, in November, hypertension had worsened and there was a gradual, progressive deterioration of renal function: creatinine was about 1.95 mg/dl and urinalysis revealed proteinuria = 50 mg/dl and microhematuria. The daily urine protein loss was found to be about 1,200 mg. Hydration was started and cabozantinib 60 mg per day continued. After 2 weeks, serum creatinine was still about 1.85 mg/dl and daily urine protein loss was 1,000 mg. Then, in agreement between oncology and nephrology specialists, cabozantinib was reduced to 40 mg per day. After 2 weeks, serum creatinine was 1.7 mg/dl, daily urine protein loss was 600 mg, and after 2 more weeks creatinine was 1.6 mg/dl and daily 24-h urine protein loss was 250 mg.

Then, the patient continued cabozantinib 40 mg per day, no more renal toxicity was observed, and the treatment was well-

tolerated. In March 2022, total body CT showed stable disease and treatment with cabozantinib 40 mg per day is still ongoing (Figure 3).

3.2.2 Nephrologist opinion

According to the advent of new oncological treatments (for example, combinations of immunotherapy–immunotherapy and TKI–immunotherapy), drug toxicity and especially renal toxicity are more frequent than before. As reported by ESMO clinical guidelines (17), for example, renal dysfunction is rare with ipilimumab and with anti-PD-1 therapies, described in <1% of treated patients (32). The incidence is higher with combination of ipilimumab plus nivolumab, reaching 4.9%, with 1.7% of grade 3 to 4 toxicity. Similarly, sequential therapy with ipilimumab followed by nivolumab is associated with a high incidence of 5.1% (33).

In order to manage different types of toxicity, a timely treatment of renal injury is crucial and in this setting the nephrologist's role is of primary importance inside the MDT.

Furthermore, a new evolving field, namely, onco-nephrology, has emerged during the last few years. It includes the vast spectrum of renal disorders that can arise in patients with cancer. A differential diagnosis between progression of underlying renal disorders and secondary disorders due to oncological treatments or to the malignancy itself is essential in order to allow the oncologist to continue the antineoplastic therapy. Cancer therapy is increasingly prescribed in elderly patients, a population often already affected by multiple morbidities and preexistent CKD (chronic kidney disease). Therefore, it is important to consider how the presence of CKD, AKI (acute kidney injury), and other renal disorders may affect treatment options and outcome and how certain therapies may increase the risk of kidney toxicity (34).

Regarding our case report, it is well known that VEGFR2 inhibitors lead to some adverse events such as proteinuria, hypertension, hand–foot syndrome, and kidney dysfunction whereas ICIs lead to other adverse events as autoimmune disorders, such as thyroiditis, colitis, skin disease, and different forms of nephritides.

Clinically, renal adverse effects of anti-VEGF therapies are arterial hypertension, proteinuria, rarely nephrotic syndrome, AKI, or CKD. Various parts of the nephron can be injured; 42% of the total number of renal adverse effects is represented by renal impairment, 47% by metabolic disturbances, and hypertension in 11% (31).

Podocytes and endothelial cells are involved, resulting in severe alteration of the architecture and function of the GBM (35).

Proteinuria is described as one of the most common renal side effects of other anti-VEGF drugs and frequently occurs with hypertension (36). It is the result of glomerular filtration barrier impairment in the glomeruli, releasing an abnormal amount of plasma proteins, mainly albumin, in urine, and it is a direct marker of therapy nephrotoxicity. The incidence and rate of proteinuria are variable, and the incidence of all grades of proteinuria during the treatment with cabozantinib was about 12%, whereas no one was with severity > grade 3. Despite this high frequency, most cases of proteinuria are asymptomatic or not severe (37–39).

Proteinuria, hypertension, and kidney injury are closely related to the destruction of the integrity of the glomerular filtration barrier, composed of podocytes, a glomerular basement membrane, and endothelial cells. TKI-induced endothelial cell damage leads to the compensatory expression of pro-angiogenic factors and the formation of an abnormal endothelial–podocyte cross talk and podocyte injury (40).

On renal biopsy from patients receiving TKIs, the most common pathological findings are thrombotic microangiopathy (TMA), MCD (minimal change disease), and FSGS (focal segmental glomerulosclerosis), as the result of direct cellular toxicity on endothelial cells and/or podocytes (40–42). In addition, some drugs can cause damage to different tubular transporters. For example, according to submitted studies the incidences of hypopotassemia and hypomagnesaemia were about 11% and 16% during the treatment with cabozantinib (38, 43).

Despite TKIs, ICIs can cause different autoimmune diseases known as immune-related adverse events (irAEs) (44). Usually, kidneys are less involved; however, up to now, ICI-associated

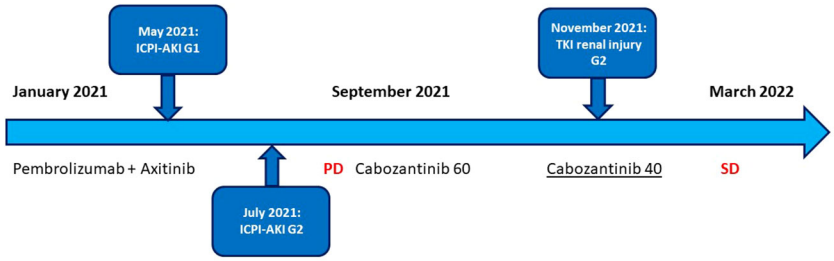

FIGURE 3
Timeline management of nephrological toxicity in course of ICI- and TKI-based treatment.

AKI (ICPI-AKI) has posed challenges in diagnosis and management (44–47). Renal histopathology mainly reveals ATIN (acute tubular–interstitial nephritis). Different causes of AKI should be considered, and these are also important to decide about treatment with steroids and/or interruptions of ICI therapy without leading to tumor spreading and/or irreversible organ damage.

A recent multicenter study identified three independent risk factors for development of ICI-AKI: 1) concomitant use of PPIs; 2) combination treatment with anti–CTLA-4 and anti–PD-1/PD-L1 agents; and 3) lower baseline eGFR (48).

According to the results of this study, patients receiving PPIs, those receiving combination ICPI therapy, and those with a lower baseline eGFR may receive closer renal surveillance.

In this setting, the figure of the onco-nephrologist is very important for the consultation and consideration of kidney biopsy, especially for patients with persistent stage 1 AKI, and those who develop stage 2 or 3 AKI.

Indeed, in a recent review (49), patients who develop stage 1 AKI treated empirically with steroids whose kidney function does not improve should undergo kidney biopsy to assess for alternative etiologies of AKI (e.g., glomerulonephritis, which may require additional immunosuppressive therapies). Patients with stage 2 or 3 AKI who have plausible alternative etiologies for AKI other than ICIs should proceed directly to kidney biopsy.

Kidney dysfunction under TKIs usually resolves with dose reduction or drug discontinuation, and it depends, in part, on the patient's baseline serum creatinine. However, these patients also present many risk factors for CKD such as diabetes, old age, hypertension, and nephrectomy which can lead to chronic kidney failure. In the KDIGO Controversies Conference on onco-nephrology, for patients with CKD, TKIs may be used at a lower-than-standard dose and then increased according to individual tolerability (50). According to international clinical guideline recommendations for the management of immune-related adverse events, including ICI-AKI, currently, there are no therapies to treat these renal complications, apart from drug discontinuation, dose reduction, or symptomatic treatment. Thus, this is really important in order to better understand the underlying mechanisms to reduce nephrotoxicity without inhibiting the anti-angiogenic effects on cancer.

Recommendations for management of ICI-associated adverse renal effect have been recently summarized in a complete review by Hermann and Perazella (51).

International guidelines suggest the following management of immune-related adverse events (15, 50, 51): in case of ICPI-AKI grade G1, the treatment with ICI can be continued; in case of ICPI-AKI grade 2, the treatment with ICPI should be suspended and restarted once serum creatinine is back to grade G1. For patients with ICPI-AKI grade 2 or more, such as the ICPI-AKI described in this case report, steroid treatment may start with prednisone 0.5–1 mg/kg/day for G2, 1–2 mg/kg/day for refractory G2 and for G3–G4.

ASCO guidelines also suggest the use of other immunosuppressive agents like mycophenolate mofetil, azathioprine, cyclophosphamide, and infliximab if corticosteroid therapy is not enough (52).

Nevertheless, in Italy these immunosuppressive agents are not recommended in immune-related adverse events and so we cannot express an opinion on this subject.

However, according to these findings, baseline renal function, urine analysis, and electrolytes are three of the most important things to monitor during cancer treatment with both TKIs and ICIs, especially in patients with comorbidities (diabetes, arterial hypertension) that can cause one of the most difficult problems for making the differential diagnosis between collateral effects of antineoplastic drugs or preexistent diseases. Therefore, a complete evaluation of kidney function prior to oncological therapies is mandatory for prolonging the survival of our patients (53) (Table 1).

3.3 Case 3: Multidisciplinary management of liver toxicity

3.3.1 Case presentation

In September 2019, a 63-year-old woman, with a past medical history of active smoking (two packs a day for the last 15 years), who experienced a progressive weight loss and dyspepsia, underwent abdominal ultrasound and CT scan which showed a huge expansive mass (10.5 × 7.8 cm) at the level of the middle and lower portions of the left kidney. She thus underwent left nephrectomy. Histopathological report diagnosed a clear cell-type RCC, grade 2 Fuhrman nuclear grading, pT2b pN0 according the AJCC TNM system. Follow-up was negative until June 2020, when a chest CT scan showed at the level of the left lung the increase of both a parascissural nodule, measuring 10 × 9 mm (previously 2.5 mm), and of a nodule in the posterobasal segment (measured 4.5 vs. 3.5 mm). She underwent atypical pulmonary resection, and the histological examination described a pulmonary localization of RCC. After 2 months, due to acute dyspnea, she underwent a further CT scan which showed left lung pleural effusion and necrotic solid tissue localized at the apex of the left lung (10 × 3.3 cm), which was infiltrating the pleura, pericardium, and fifth rib; additional pleural implants; carcinomatous lymph nodes; and mediastinal lymphadenopathies. The patient then had blood tests which showed levels within a normal range. According to the IMDC, for Karnofsky Performance Status below 80%, she was categorized prognostically as at an intermediate risk. Consequently, from January to May 2021, she underwent administration of six cycles of pembrolizumab + axitinib with partial radiological response, and her global clinical conditions improved. In May

2021, after the sixth cycle, routine biochemical tests showed an increase in serum transaminases, with a normal bilirubin value: the glutamic-oxaloacetic transaminase (GOT) value was 83 U/l (normal value ≤32 U/l) whereas the glutamic-pyruvate transaminase (GPT) was 142 U/l (normal value ≤33 UI/l). According to the classification NCI-CTCAE (v.5.0) (54), the patient had a grade 2 liver toxicity. As soon as the increase in transaminases was detected, a hepatological consultation was requested. Other potential causes of liver toxicity were ruled out (e.g., viral, autoimmune, alcohol, use of medications, supplements, or herbal products); no other alteration of liver tests (e.g., total bilirubin, alkaline phosphatase, gamma glutamyl transferase (GGT), coagulation tests, electrophoretic protidogram) was detected. The patient did not report any abdominal complaint, and physical examination did not show either hepato- or splenomegaly. Ultrasound did not detect liver metastases. According to the analyzed values (Table 1), an immune-related liver toxicity was diagnosed. Both drugs were stopped, and according to the current guidelines, oral steroids (prednisone, 1 mg/kg/day) were started. Follow-up biochemistry performed after 2 weeks of steroid treatment showed normal liver tests (GOT 16 U/l; GPT 30 U/l), and prednisone was then progressively tapered and stopped. Thanks to the help of the hepatologist and medical therapy, liver toxicity quickly resolved, and the patient resumed the scheduled treatment with pembrolizumab and axitinib at a reduced dosage of 3 mg daily bid, which is currently ongoing with good compliance and clinical results (Figure 4).

3.3.2 Hepatologist opinion

During ICI monotherapies (such as ipilimumab, nivolumab, and pembrolizumab), liver enzymes' increases in various orders of magnitude have been reported to cardiological toxicityoccur in around 2%–10% of patients (1). In case of combination treatments, these increases tend to occur more frequently, with figures as high as 25%–30% of all grade toxicities in the case of ipilimumab and nivolumab, whereas incidence of G3 toxicity occurrence is more limited, at an approximate 15% incidence rate (52, 55, 56). Anti–PD-1/PD-L1 seems to have a lesser incidence of liver-related IrAEs of any grade as compared with anti–CTLA-4 (1, 3). Liver failure with encephalopathy in the context of acute fulminant hepatitis remains instead a rare evidence, occurring in 0.4%–0.14% of treated patients (56). The onset of liver enzyme alterations usually develops within the first 6–12 weeks after treatment initiation (57); even with some discordances, some authors have suggested different timings of onset for the different ICIs (anti-CTLA-4 vs. anti-PD-1 vs. anti-PD-L1) (17, 56) and an earlier occurrence of adverse events in case of combination treatments as compared with monotherapies (56). Data on ICI retreatment after an episode of drug-related liver adverse events are very poor. In a large retrospective study, among patients who had resumed ICI treatment after transaminase decrease after temporary drug discontinuation, 26% of them developed recurrence of hepatotoxicity (58).

Liver-related adverse events (LRAEs) occurring during ICI treatment are usually reported and scored according to the CTCAE (54). It has been recently suggested that the preferred term to denominate cases of liver injury caused by ICIs should be "immune-mediated liver injury caused by immune checkpoint inhibitors" (ILICI) (56). However, in a more hepatological perspective, we suggest to first define the pattern of liver

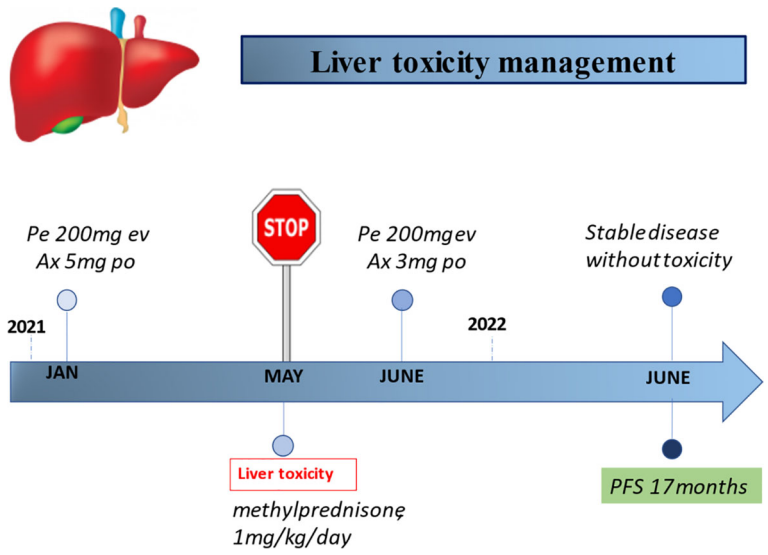

FIGURE 4
Timeline of the management of liver toxicity during ICI-based treatment. Pe, pembrolizumab; Ax, axitinib.

enzyme elevation by calculating the ratio of serum alanine amino transferase (ALT) to alkaline phosphatase (ALP) levels (R value = [ALT/upper limit of normal (ULN)]/[ALP/ULN]), which allows to categorize the event as hepatocellular (R >5), mixed (R >2 to <5), or cholestatic (R <2). Different R patterns may help in characterizing the observed adverse event and help to distinguish which drug is more probably involved in determining it. Nevertheless, it should be underlined that LRAEs are only imperfectly described by defining the increase in either hepatocellular or cholestatic indexes. Evaluation of liver damage severity must also include check of liver synthetic function, as expressed by laboratory parameters such as coagulation (prothrombin time/international normalized ratio), and total serum bilirubin, and by the presence of hepatic encephalopathy/ascites on the bases of, respectively, clinical and ultrasound findings; in fact, acute hepatitis is considered severe if the INR is >1.5, bilirubin is elevated (usually >2X ULN), and fulminant (i.e., potentially leading to hepatic failure), if impaired coagulation is accompanied by hepatic encephalopathy and/or prolonged jaundice and/or onset of ascites (59).

At present, liver biopsy should be considered for patients with more severe liver toxicity (grade >3) or in case of uncertain diagnoses. Patterns of liver toxicity during ICI treatment are still currently scarcely characterized from a histological standpoint (56), and biopsy of the liver might be useful to optimize the management in elusive cases of persistent/refractory LRAE, if blood tests or imaging evaluation does not provide conclusive information. In addition, to avoid misnomer, it has been pointed out that until a larger histological database of patients with suspected ILICI will be available, the term "hepatitis" should only be reserved for patients who have histological findings consistent with this entity. Also, in case of a prevalent cholestatic serum pattern, terms such as "cholangitis" should be avoided and only reserved for those who have either supportive histological findings or results of other reliable diagnostic tests (59).

Interestingly, ICI-induced liver toxicities do not display the histological (e.g., lack of plasma cells) and serological (absence of autoantibodies) features of an autoimmune hepatitis (AIH), but this notwithstanding, the current pharmacological management is mainly based on protocols derived from those used in the treatment of this liver disease (56). As recently reviewed (52), the mainstay of treatment is based on the use of either oral or intravenous steroids in various dosages and dose-escalation protocols, whereas the use of other immunosuppressant commonly employed in the management of AIH, such as oral mycophenolate mofetil, still needs further proof of efficacy and safety. In this setting, the use of infliximab is contraindicated for the concerns regarding its intrinsic hepatotoxicity. Furthermore, it should be underlined that liver transplant, as an option for the management of ICI-induced liver failure, is unfortunately not considered, since patients are affected by malignant tumors.

3.4 Case 4: Multidisciplinary management of cardiological toxicities

3.4.1 Case presentation

A 74-year-old woman, due to persistent cough and abdominal pain on the left side, underwent an abdominal ultrasound and a total body CT scan that showed a mass of 2 cm in the left kidney, suspected for primary tumor, and multiple nodular lung lesions. After 1 month, in December 2020, the patient underwent a left radical nephrectomy, whose histological examination revealed a clear-cell type RCC (pT3a pNx according to the AJCC 2017 classification, 8th edition). After 2 months, the patient was referred to our center for the first oncological evaluation. The past medical history comprised systemic hypertension treated by angiotensin II receptor blockers and paroxysmal atrial fibrillation on direct oral anticoagulants (DOACs), allergic asthma on foster therapy, and type 2 diabetes mellitus treated by metformin. An echocardiogram showed a normal bi-ventricular dimension, wall thickness, and systolic function. The patient was in good general condition, the blood pressure was within the normal limit, the blood test showed normal renal function, and electrolytes were within limits, without proteinuria. According to the IMDC intermediate risk, a combination therapy with pembrolizumab and axitinib was started. The patient was instructed to monitor the blood pressure at home and to contact the clinic in case of hypertension or any new symptoms. After the third cycle of therapy, the patient reported asthenia and headache. The blood pressure was increased (180/90 mmHg). The patient was referred to a cardiologist. The electrocardiogram showed a sinus rhythm with a heart rate 90 bpm without repolarization changes. The blood pressure was persistently increased. Troponin showed a negative result. Echocardiogram showed a normal bi-ventricular systolic function with FEVS 61%. Antihypertensive therapy was implemented with the addition of amlodipine with good response to therapy. Furthermore, due to the drug interference between amlodipine and metformin by pharmacodynamic antagonism, the patient was closely observed for the risk of hypoglycemia.

After 4 months, a total body CT scan was performed, showing stability of the disease. Blood test showed kidney function, electrolytes, and glucose levels within the normal limits, and no proteinuria. Cardiovascular evaluation showed normal ECG and normal blood pressure (140/80 mmHg). The patient was asymptomatic. Therefore, considering the stability of the disease, the results of the laboratory tests, the cardiovascular evaluation, and the improvement in symptoms, the patient continued the scheduled therapy, which is still ongoing with good tolerability (Figure 5).

3.4.2 Cardiologist opinion

In this case, a combination of two different classes of agent were administered to the patient. Each agent holds the potential to determine different cardiovascular toxicities. One of the most

common adverse reactions of axitinib is systemic hypertension. A meta-analysis including 77 studies showed that arterial thromboembolism, cardiac ischemia and cardiac dysfunction rate among cardiotoxic effect, and hypertension were the most common and clinically recognized adverse events with OR 5.28 [4.53–6.15] (60). Another meta-analysis showed that the risk of hypertension with axitinib was substantially higher than other approved VEGFR-TKIs. In addition, the risk of all-grade and high-grade hypertension associated with axitinib is significantly higher in RCC than that in non-RCC (61).

Generally, hypertension is an established risk factor for chemotherapy-induced cardiotoxicity, and poorly controlled blood pressure can influence outcomes for cancer patients. Therefore, continuous monitoring and medical treatment with antihypertensive agents are recommended for axitinib-associated hypertension. There is no general consensus on the best modality for blood pressure monitoring. The AIOM (Associazione Italiana di Oncologia Medica-Italian Association of Medical Oncology) guidelines recommend a weekly based monitoring in the first 8 weeks and specifically a blood pressure measurement before every cycle. In addition, guidelines recommend to obtain a good blood pressure before starting treatment (62). The ESMO (European Society of Medical Oncology) recommend generally more frequent BP monitoring in those patients with preexisting hypertension and known to be at higher CV risk. Once stable blood pressure is achieved, the evaluation schedule might be aligned with home BP monitoring or routine clinical evaluations, at least every 2–3 weeks for the remainder of the treatment (63). In this specific case, the patient had history of systemic hypertension and was already on treatment. Home monitoring was then recommended. Despite that increased blood pressure is usually reported after the first dose of treatment, in this case it was observed after the third dose. Therapy combination was used to reach a good blood pressure profile (calcium antagonist was added to angiotensin II receptor blockers).

The second agent administered to the patient was the ICI pembrolizumab. This agent can be associated with a spectrum of adverse effects mainly related to irAEs and can affect multiple organs including the cardiovascular system. Although rare, cardiovascular IrAEs can be fulminant (64). A recent meta-analysis including multiple sources (World Health Organization, WHO pharmacovigilance database with more than 16,000,000 adverse drug reactions, 16 international multi-institutional treatment data, and all published clinical trials to characterize more than 750 fatal irAEs) reported that ICI-associated toxic effects are rare and occur very early after therapy initiation and with marked distinctions between ICI regimens. Combination therapy had more frequent multiorgan involvement, and nearly one-third of all deaths were from myocarditis, myositis, and/or neurologic events (55). Combined PD-1 plus CTLA-4 blockade triggers substantially more irAEs than anti–PD-1 alone (55%–60% vs. 10%–20% high-grade events) (65, 66). Notably neurologic and cardiac toxic effects comprised nearly half of deaths. Many of these cardiological adverse events are often unrecognized until they are severe and potentially fatal. AIOM guidelines suggest to perform an electrocardiogram before treatment. Serial troponin measurements are not recommended as evidence currently does not support their use (67). Troponin evaluation may be considered in those patient candidates to treatment with combination of ICIs known to be more toxic. In case of high-

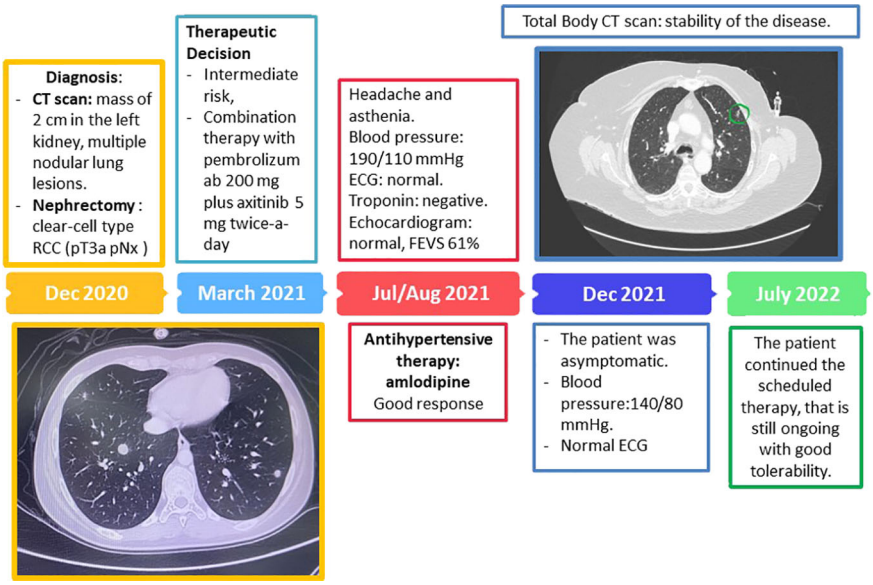

FIGURE 5
Timeline management of cutaneous toxicity in the course of cabozantinib treatment.

risk treatment, troponin should be repeated at 2, 4, and 12 weeks. Advanced cardiovascular imaging, such as strain analysis by echocardiography (68) and cardiovascular magnetic resonance (CMR) (69), seems a promising tool to detect toxicities and predict outcome, but data are limited and they are not recommended at this stage. Both AIOM and ESMO guidelines recommend, in case of symptoms, to perform comprehensive cardiological evaluations including electrocardiogram, troponin and NT-pro BNP (brain natriuretic peptide) evaluation, echocardiogram with strain analysis, and cardiovascular magnetic resonance (CMR), in case of symptoms and/or troponin level rise and/or ECG changes (5, 62). Of note, guidelines suggest to consider, in addition to chest pain and dyspnea, symptoms such as fatigue and asthenia. Endomyocardial biopsy should be considered if the diagnosis is highly suspected with an otherwise negative workup and/or the patients cannot undergo non-invasive assessment due to hemodynamic instability (17, 70). In this case, the patient was treated with single ICI and serial troponin and/or ECG were then not recommended. She did not present with any toxic effect from pembrolizumab. Troponins and echocardiogram were performed when the patient complained of atypical symptoms, but these did not reveal any abnormalities. In the clinical case presented, we concluded that only axitinib determined the increased value of blood pressure. Therefore, antihypertensive therapy was implemented with a good response preventing further increases.

Anticancer therapies utilized in GU cancer can have cardiac-related toxicities, and the collaboration between oncologist and cardiologist is crucial. One of the priorities of the cardio-oncology field is the possibility to improve the cardiovascular screening to mitigate risk factors for cardiotoxicity prior to the beginning of treatment and to identify high-risk patients requiring a closer follow-up (Table 1). The goal is to avoid cancer therapy interruption and to prevent cardiovascular events.

3.5 Case 5: Multidisciplinary management of cutaneous toxicities

3.5.1 Case presentation

A 60-year-old male patient with mRCC treated with cabozantinib was referred to our department. His personal history showed type 2 diabetes mellitus, hypertension, and atrial fibrillation.

In July 2012, he underwent surgery of left nephrectomy and histological examination showed renal clear cell carcinoma, pT2a. Therefore, the patient started clinical and radiologic follow-up.

In July 2016, the total body CT scan showed a local relapse of disease and distant metastases, located in the paravertebral muscles, right gluteus muscle, bones, and lungs. In August 2016, the patient underwent a biopsy of the gluteus muscle, which confirmed the diagnosis of metastases from RCC. Therefore, he started first-line therapy with sunitinib 50 mg per day 4 weeks on/2 weeks off, from September 2016 to June 2017. Then, the total body CT scan showed a disease progression to the lungs and muscles. Considering the previous treatment, the patient started therapy with nivolumab and in July 2017 he underwent radiotherapy for muscular metastases (paravertebral and gluteus) and stereotactic radiotherapy on a lung metastasis in July 2020. Nivolumab was administered until February 2021, when the total body CT scan showed a disease progression on the liver and pancreas.

At this point, in April 2021, the patient started a third-line treatment with cabozantinib 60 mg daily. After 28 days, at the beginning of the second cycle of therapy, the patient reported erythema on the dorsal hands, not associated with pruritus. However, we decided to continue the therapy. One month later, at the beginning of the third cycle, we found a worsening of cutaneous toxicity, with lesions resembling cigarette burns (Figure 6).

At this point, we asked a dermatologic consultant, in order to evaluate and treat these lesions. Since lesions were limited to the upper arms, with less than 30% of skin involved, and Nikolsky sign showed a negative result, we considered this skin eruption as prurigo-like and our dermatologist prescribed azithromycin 1 cp/day for 3 days, fluorescein lotion, silver nitrate gel, cicatrizin gel, zinc oxide, delicate hand cleanser, and nitrile gloves. The patient started this treatment without discontinuation of cabozantinib. At the subsequent visits, the patient showed a reduction in the skin toxicity (Figure 6), up to a total regression of the lesions on the hand in July 2021.

However, in August 2021, due to grade 3 gastrointestinal toxicity and an episode of syncope with hypotension, we discontinued treatment with cabozantinib, and a few months later, in October 2021, we decided to resume the cabozantinib at a lower dosage (40 mg).

The total body CT scan, performed in November 2021, showed a partial response of the disease. The patient is still under treatment, with no other severe toxicity.

3.5.2 Dermatologist opinion

Cutaneous adverse events may occur frequently with the use of cabozantinib (71). Cabozantinib is a multi-tyrosine kinase inhibitor (TKI), with activity against MET, RET, AXL, VEGFR2, FLT3, and c-KIT (72). In the METEOR trial, cabozantinib showed a better median PFS and OS versus everolimus in patients who progressed after a previous line with an anti-VEGFR TKI (38, 73). The most frequent adverse events with cabozantinib were diarrhea, fatigue, hypertension, stomatitis, nausea, and hand–foot syndrome.

The hand–foot syndrome (palmar–plantar erythrodysesthesia) is a potentially painful dermatological condition, reported by 43% of the patient in the METEOR trial. The mechanism by which HFS develops is not fully understood; it is possible that the drug interferes with pericyte-mediated endothelial survival mechanisms, leading to damage to the capillary endothelium in

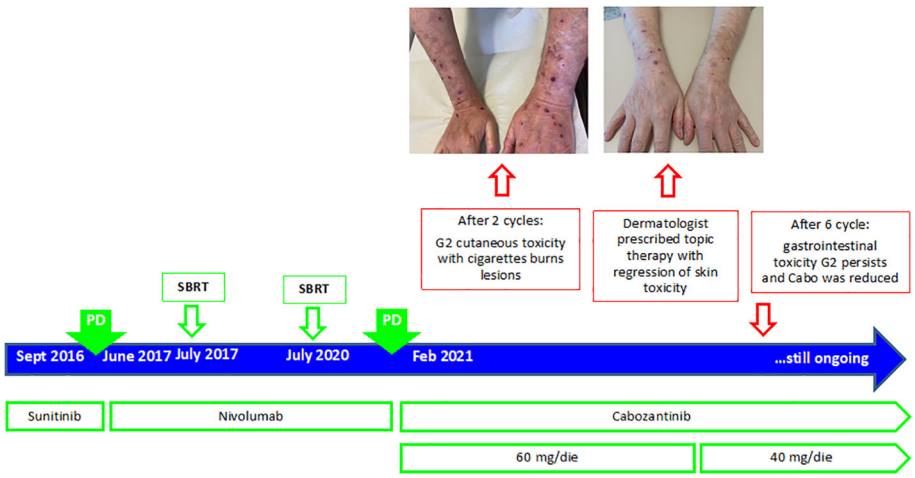

FIGURE 6
Timeline management of cardiovascular toxicity in the course of ICI- and TKI-based treatments.

the hands and feet (74) or the inhibition of KIT (strongly expressed in the ductal epithelium of eccrine glands) (75). Prophylactic measures include pedicure to remove hyperkeratosis, use of emollients, topical exfoliation, and protection of pressure-sensitive areas. For low-severity cases of HFS, the use of urea cream and clobetasol cream, and analgesics if pain control is needed, may be sufficient to manage the AE (74). Urea cream is recommended as a prophylactic measure with usage from the first day of cabozantinib treatment (76). In our patient, skin involvement was less than 30%; therefore, we decided to continue treatment (77). Overall, the skin toxicity may be due to three different mechanisms of action: immunologic, direct toxicity, or idiosyncrasy. Some skin reactions may also be due to the patient's comorbidity and drug interaction (78). In the case of ICI-based treatment (anti-CTLA4 and anti-PD1/PD-L1), the reinvigoration of the antitumor T-cell response and the enhanced immunologic activation may result in a variety of autoimmune-like or inflammatory side effects, which can involve almost any organ system, including the skin one (79). Dermatologic complications affect between 30% and 50% of patients on ICIs, and generally, they occur as the earliest events among all irAEs. The most widely reported skin toxicities are maculopapular rash, pruritus, lichenoid eruptions, and vitiligo (80). Although they are most frequently mild and manageable, they significantly impair patients' quality of life and could lead to treatment interruption. Also, life-threating conditions like Stevens–Johnson syndrome/toxic epidermal necrolysis and the drug reaction with eosinophilia and systemic symptoms (DRESS) may occur (80). In order to avoid severe reactions that can even be lethal for the patient, it is really important to make the right diagnosis very quickly, taking into account appearance and timing and skin involvement, to understand the best pretreatment and or desensibilization, to avoid oncologic treatment discontinuation, and to obtain the best efficacy, a high compliance, and the best quality of life (81, 82). In case of severe reactions (G3–G4 cutaneous toxicity, with diffused eruption), systemic corticosteroids, withholding ICIs, and skin biopsy to exclude other causes and verify the grade of epidermic necrosis should be recommended. ICIs may be reintroduced after the resolution of cutaneous signs (67). However, it depends on clinical evolution. In our case report, the cutaneous toxicity was G2 grade; thus, skin biopsy was not done. A multidisciplinary approach is mandatory in order to create guidelines, considering that each patient is different and can have different reactions; thus, skin toxicity can be cumulative and not predictable in advance. Periodical follow-up, as well as education to an appropriate lifestyle and habits (oncosupportive care: sun protection, emollients, specific shower gel, ideal socks, avoiding aggressive products, etc.) to take care of the skin as a possible indicator of internal disease, is mandatory (83, 84). An appropriate symptomatic and etiologic (when it is possible) treatment is the better strategy for a correct balance. Probably in the future, a genetic analysis will be able to predict personal predisposition and will allow to define personalized treatment, and oncosupportive dermatology will be accepted in each oncologic team. Few biological markers such as rheumatoid factor (RF) greater than 15 IU/ml at baseline and the presence of an HLA-DRB1*11:01 genotype are emerging as potential predictive biomarkers of skin toxicity, especially in case it is associated with pruritus, in patients treated with ICI-based treatment (85, 86). However, further study will be necessary to draw up a detailed algorithm of skin care prevention in mRCC patients to improve the patients' compliance for both immunological and targeted drugs.

4 Discussion and conclusion

Today, most patients with mRCC receive systemic therapy that is ICI- or target-based, alone or in combination with each other, and may develop drug-related symptoms of different grades of severity. With the introduction of novel combinations, there was a dramatic improvement in the outcome of mRCC patients but also the occurrence of adverse events more difficult to manage, as compared with those observed with the previously used TKIs. Furthermore, due to the relatively recent introduction of these combinations in clinical practice, their cumulative dose adverse effects are still unknown. Furthermore, as the immunotherapy may affect any organs, related toxicities are often misunderstood, before becoming from moderate to severe. A prompt recognition and management of these toxicities represents a fundamental issue in oncological clinical practice, since it correlates with the outcome of cancer patients. Although both European and Italian guidelines give well-established protocol to treat immune-related toxicities according to different grades of severity (5, 67), a specific protocol to prevent the risk of developing an adverse event that may lead to a discontinuation of treatment or a dose reduction for mRCC patients has not yet been established.

In this context, MDT evaluations should be provided in any cancer center, especially for those patients, not only the most elderly and fragile, who should be investigated for preexisting unhealthy conditions, which may require a prompt support to finalize their treatment' program.

In the present paper, we reported a case series of critical toxicities that occurred in our center during treatment for mRCC and a literature review, with the aim of supporting the MDT's role in genitourinary cancer care. Indeed, the different specialized disciplines integrated in the genitourinary MDT have demonstrated to help oncologists by providing a better care to mRCC patients, mainly during treatment and follow up. Joining the efforts from different healthcare professionals improves patient management, by an early recognition of treatment side effects and relief of severe symptoms that may occur during treatment with both immune- or target-based therapy. In this way, by preventing and reducing drug-related adverse events, patients' quality of life as well as adherence and compliance to therapies became better (87). According to other complex solid tumors like head and neck cancer (88), we conclude that a comprehensive evaluation and monitoring of mRCC patients by specialized MDTs is strongly recommended to improve treatment adherence and tolerance, reduce long-term side effects, improve quality of life, and ultimately improve treatment outcome and survival.

Author contributions

MR and MP write the manuscript. All the authors revised and approved the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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