Innovative Approaches and Future Horizons in Renal Cell Carcinoma Immunotherapy

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I. Introduction

Cancer is a group of diseases characterized by the abnormal and uncontrollable division of cells, primarily caused by genetic mutations that alter the way normal cells function. The immune system is the body's defense mechanism, identifying and eliminating destructive pathogens, particularly any possible cancer cells. The immune system should recognize and destroy cancer cells by detecting any seemingly irregular antigens, thereby initiating an immune response. However, cancer cells avoid these mechanisms through various methods, such as building an immunosuppressive environment and expressing immune checkpoint proteins that constrain immune activity. Immunotherapy is a type of treatment to strengthen the body's immune response to fight cancer effectively. It has proven beneficial in subduing the cancer's immune evasion methods through targeting and balancing immune checkpoints. In the following review, we will explore how renal cell carcinoma (RCC) evades the immune system and the different methods of immunotherapy utilized to treat it. We will review specific immune cells being targeted, the treatments' efficacy, the restrictions, and the FDA-approved options available. We will also examine relevant clinical trials, including CheckMate 025 (NCT01668784) and CheckMate 214 (NCT02231749), focusing on the upcoming innovations regarding immunotherapies in RCC.

II. Overview

As the most prevalent malignancy within the kidney, accounting for 2-3% of all cancers, RCC has increasingly displayed promising responses when reacting to immunotherapy treatments. RCC begins from the lining of the proximal convoluted tubule, otherwise known as a part of the kidney involved in filtering urine and blood production. While several factors can contribute to the development of RCC, smoking is a primary risk factor that has doubled the likelihood of possibly being diagnosed with this disease. Obesity, exposure to hazardous chemicals such as asbestos, long-term use of diuretics like thiazides, and chronic urinary tract infections all elevate the risk of developing RCC (Escudier 2012). This disease's global impact has notably increased from when it was first discovered, which could potentially be attributed to improved radiological techniques that detect tumors during evaluations regarding an unrelated condition. RCC is diagnosed twice as often in men compared to women; the highest outcome observed in patients was in their 60s. Within RCC development, genetic mutations are significant in the von Hippel-Lindau (VHL) gene being predominant in previous clear-cell RCC cases (Bleumer 2003). Leading to the dysregulation of factors inducing hypoxia, these mutations promote tumor growth and angiogenesis; other important mutations include those in the *PBRM1*, *BAP1*, and *SETD2* genes, altering the remodeling of chromatins, cell cycle regulation, and DNA repair mechanisms. The genetic abnormalities studied add to the aggressive nature of RCC and its propensity for metastasis. When the VHL gene loses its inability to function, it results in the stabilization of hypoxia-inducible factors (HIFs). The importance of the VHL pathway in RCC pathogenesis is shown through the activation of the transcription of genes involved in cell proliferation, angiogenesis, and survival (Considine 2019). Early-stage RCC limited to the kidney has a five-year survival rate of approximately 90%.

However, metastatic RCC (mRCC) has a much worse outcome, with a five-year survival rate of less than 10%, specifically due to the cancer being spread to other parts of the body. Patients with renal cell carcinoma are usually treated with a combination of surgical, systemic, and immunotherapeutic methods. Surgical interventions, such as nephrectomy, serve as the primary solution for localized RCC, aiming to remove the tumor and area of the affected kidney tissue. Regarding mRCC, traditional chemotherapy and radiotherapy have been resolved as ineffective, with response rates being 6% (Sheng 2019). Consequently, immunotherapy has become a critical component in treating mRCC. Non-specific cytokine therapies, including interleukin-2 (IL-2) and interferon-alpha (IFN-α), have shown a variation of positive results, yet there has been significant toxicity. Recent innovations have introduced checkpoint inhibitors, such as nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4), blocking inhibitory pathways to aim for an immune response against cancer cells. These agents have improved survival rates in clinical trials and are now standard treatments for mRCC. Combination therapies are being studied, such as how tyrosine kinase inhibitors like sunitinib with checkpoint inhibitors are currently being tested.

III. Overview of immunotherapies used in RCC

RCC uses several mechanisms to evade the immune system to create a tumor microenvironment that supports its growth and metastasis. One of the key methods is the alteration of immune checkpoint pathways. Usually, these pathways help stabilize self-tolerance, measuring the duration and amplitude of physiological immune responses. However, RCC tumors exploit these pathways, specifically by overexpressing programmed death-ligand 1 (*PD-L1*) and *PD-L2*, which bind to the *PD-1* receptor on T cells. This interaction inhibits T cell activation and allows the tumor cells to avoid immune detection and destruction (Bleumer 2003). Another important mechanism is the dysregulation of the von Hippel-Lindau gene - a gene commonly mutated in RCC. The lack of VHL presence leads to the accumulation of hypoxia-inducible factors (HIFs), promoting angiogenesis and creating a hypoxic tumor microenvironment. Hypoxia can further constrain immune cell function and support the gathering of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which suppress the anti-tumor immune response (Deleuze 2020). Tregs delay the activation and proliferation of effector T cells, thus undermining the immune response against the tumor. MDSCs inhibit T cell function through the production of reactive oxygen species (ROS) and nitric oxide (NO), providing an overall contribution to immune evasion (Escudier 2012). In RCC, multiple immune cell types are often dysregulated. Tumor-infiltrating lymphocytes (TILs) are frequently seen in RCC tissues, yet their functional capacity is often impaired. Cytotoxic T lymphocytes (CTLs) are crucial to target and kill tumor cells, often being ineffective due to the immunosuppressive tumor microenvironment. Natural killer (NK) cells are another important factor regarding the anti-tumor immune response, as they also have reduced activity in RCC (Condosine 2019). Tumor-associated macrophages (TAMs) can lead to either pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, which are also dysregulated in RCC. The tumor microenvironment frequently skews TAMs towards an M2 phenotype, corroborating tumor growth and suppressing the anti-tumor immunity response. The exchange between these immune cell types and the tumor microenvironment builds a network that supports progression in RCC treatment responses and metastasis.

RCC has been treated with several forms of immunotherapy and has shown considerable promise, especially for advanced or metastatic cases. The types of immunotherapy used for RCC include cytokine-based therapies, immune checkpoint inhibitors, and combination therapies. One of the earliest forms of RCC immunotherapy was cytokine-based therapy, specifically interleukin-2 (IL-2) and interferon-alpha (IFN-α). IL-2 is a potent cytokine that stimulates the proliferation and activation of T cells and natural killer (NK) cells, targeting and destroying cancer cells. These therapies block inhibitory pathways that obstruct the immune response, shaping the body's capacity to fight the cancer. The most significant checkpoint inhibitors used in RCC target the PD-1 receptor and its ligand PD-L1, along with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Nivolumab, an anti-PD-1 antibody, was approved for mRCC treatment and has shown many improvements in survival rates in comparison to standard treatments (Deleuze 2020). Ipilimumab, an anti-CTLA-4 antibody, is also used in combination with nivolumab for synergistic effects, providing increased improvement in patient outcomes. Researchers have increasingly focused on combination therapies for the treatment of RCC. Combining immune checkpoint inhibitors with other treatments such as TKIs has depicted enhanced efficacy. For example, the combination of nivolumab and ipilimumab with TKIs such as sunitinib or cabozantinib has resulted in better response rates and delayed progression-free survival compared to monotherapies (Considine 2019). These combinations utilize the complementary mechanisms of action of checkpoint inhibitors regenerating the immune system, with TKI target pathways being crucial for angiogenesis and tumor growth. Another recent approach in RCC immunotherapy includes the use of vaccines and adoptive cell transfer therapies. The goal of cancer vaccines is to stimulate the immune system to attack RCC cells specifically. Vaccines targeting tumor-associated antigens such as carbonic anhydrase IX (CAIX) have shown positive results in early clinical trials. Another option that is being explored is adoptive cell transfer, involving isolating and expanding tumor-infiltrating lymphocytes (TILs) from the patient and reinfusing them after *ex vivo* activation (Escudier 2012).

RCC immunotherapies target a group of immune cells, including CTLs, NK cells, dendritic cells, and indirectly Tregs and MDSCs - these therapies' goal is to restore and develop the anti-tumor immune response to help patients diagnosed with advanced RCC. CTLs have proven to be vital to the immune response against cancer, as they recognize and kill cancer cells through the identification of tumor antigens presented by major histocompatibility complex (MHC) molecules. In RCC, CTLs usually become dysfunctional because of the immunosuppressive tumor microenvironment. Immune checkpoint inhibitors, such as nivolumab and ipilimumab, work by blocking the inhibitory signals that harm proper CTL function. By inhibiting these checkpoints, these therapies revive CTLs, aiding them to effectively target and destroy RCC cells (Sheng 2019). NK cells are essential to the anti-tumor immune response and unlike CTLs, NK cells can kill tumor cells without previous sensitization to specific antigens. They identify the stressed cells in the absence of antibodies and MHC which makes them more effective against a wide range of cancer cells. NK cell activity is allocated by a balance of activating and inhibitory receptors. In RCC, the tumor microenvironment can damage NK cell function, meaning immunotherapies, such as cytokine-based treatments with IL-2, can improve cytotoxic NK cell activity (Bleumer 2003). DCs are crucial to initiate and regulate immune responses by displaying antigens to T cells. In RCC, dendritic cell function can be impaired, resulting in inadequate T cell activation. Experimental immunotherapies can refine dendritic cell function, improving antigen presentation. For example, dendritic cell vaccines are being tested

to boost the immune response by loading DCs with tumor antigens and reinstalling them in the patient to stimulate a healthy T cell response (Rini 2019). Tregs and MDSCs are indirectly targeted by immunotherapies due to their role in suppressing the immune response. Tregs restrain the function of effector T cells and maintain immune tolerance, which can be harmful to cancer. Targeting these immunosuppressive cells means immunotherapies can reduce their inhibitory effects, strengthening the immune response against RCC. For example, strategies may include depleting Tregs or impeding their function to amplify the antitumor activity of effector T cells (Deleuze 2020). More specific combination therapies have also been developed to target numerous immune cell types simultaneously. This includes combining immune checkpoint inhibitors with agents that target angiogenesis, thus regulating the tumor microenvironment and improving the infiltration and function of immune cells; this approach has been successful in improving treatment outcomes for RCC patients (Considine 2019).

Clinical research has demonstrated the efficacy of immunotherapy in patients with advanced or mRCC. Immunotherapy, including cytokine-based therapies and immune checkpoint inhibitors, has led to positive outcomes in improving patient conditions, though its effectiveness varies depending on the type of therapy, patient characteristics, and the presence of predictive biomarkers. Immune checkpoint inhibitors have advanced RCC treatment, with nivolumab being extensively studied in mRCC. The CheckMate 025 trial demonstrated nivolumab's superiority in improving survival compared to everolimus, the standard mRCC treatment at the time (NCT01668784). Patients treated with nivolumab had a median overall survival of 25 months, compared to approximately 19 to 20 months for those treated with everolimus (Deleuze 2020). Additionally, the objective response rate (ORR) was significantly higher with nivolumab, highlighting its ability to achieve durable responses in certain patients. The combination of nivolumab and ipilimumab has shown even greater efficacy in treating mRCC. The CheckMate 214 trial compared this combination to sunitinib, a TKI, and found that combination therapy significantly improved overall survival and response rates (NCT02231749). Patients receiving the combination therapy had a median overall survival that was not reached at the time of analysis, compared to 26 months for those on sunitinib (Considine 2019). The ORR was 42% for the combination therapy and 27% for sunitinib, indicating a higher likelihood of disease control and tumor shrinkage with the combination.

Although immunotherapy has significantly transformed RCC treatment, several challenges remain, including variability in patient response, immune-related adverse events, cost and accessibility issues, and the need for predictive biomarkers to guide therapy. A primary limitation of RCC immunotherapy is the variability in patient response; not all patients experience significant benefits from immunotherapy, and some may not respond at all. For example, while checkpoint inhibitors like nivolumab and ipilimumab have shown impressive results in clinical trials, a substantial proportion of patients do not achieve durable responses (Deleuze 2020). The reasons for this variability are multifactorial, involving factors such as tumor biology, the presence of immunosuppressive cells within the tumor microenvironment, and individual patient conditions. Immune-related adverse events (irAEs) are another significant limitation of immunotherapy. These adverse events result from the activation of the immune system and can affect various organs, leading to conditions such as colitis, hepatitis, pneumonitis, and endocrinopathies. While most irAEs are manageable with timely medical treatment, they can be severe and, in some cases, life-threatening. The management of irAEs

requires meticulous planning, along with frequently needing the use of immunosuppressive medications like corticosteroids, which can diminish the benefits of immunotherapy (Considine 2019). Immunotherapy treatments, specifically immune checkpoint inhibitors, are expensive, which can limit access for patients, especially in low- and middle-income countries. The high cost of these therapies places a large financial burden on healthcare systems and patients, potentially affecting the overall accessibility of these life-saving treatments (Sheng 2019). To have successful immunotherapy outcomes, the presence of predictive biomarkers is necessary. Currently, there are no universally accepted biomarkers that can reliably predict which patients will benefit from immunotherapy. While PD-L1 expression and tumor mutational burden (TMB) are being investigated as potential biomarkers, their utility in clinical practice is still under evaluation. The identification of reliable biomarkers would enable personalized treatment approaches, reducing the likelihood of ineffective treatments and improving patient outcomes (Rini 2019). Despite the impact of immunotherapy on RCC treatment, limitations such as variability in response, immune-related adverse events, cost and accessibility, and the need for predictive biomarkers are still prevalent, and need to be addressed.

There are several FDA-approved immunotherapies for RCC treatment, including immune checkpoint inhibitors, cytokine-based therapies, and combination approaches that have had positive results when tested in clinical trials and have been granted regulatory approval. Nivolumab is an anti-PD-1 immune checkpoint inhibitor that has been approved for the treatment of advanced RCC. It was granted approval based on the outcome of the CheckMate 025 trial, which proved to be better than Everolimus in improving overall survival and objective response rates in patients with previously treated mRCC (NCT01668784). Nivolumab works by blocking the PD-1 receptor, activating T cells, and generating an anti-tumor immune response (Deleuze 2020). Ipilimumab is an anti-CTLA-4 immune checkpoint inhibitor that has been approved for use in combination with nivolumab. The combination therapy was approved based on the CheckMate 214 trial, which showed improved overall survival and response rates compared to sunitinib, a standard treatment for mRCC (NCT02231749). Ipilimumab blocks the CTLA-4 receptor, being another method of immune suppression used by tumors (Considine 2019). The combination of immune checkpoint inhibitors along with TKIs or other immunotherapies is actively being researched. For example, the combination of nivolumab and ipilimumab shows a new way to treat RCC by utilizing the synergistic effects of multiple therapies. These combinations' purpose is to build the immune response and improve clinical results, yet they still need careful consideration of any possible side effects (Sheng 2019). The FDA has approved several immunotherapies for treating RCC, including using immune checkpoint inhibitors such as nivolumab, ipilimumab, and cytokine-based therapies like high-dose IL-2. These therapies have depicted high outcomes when tested in clinical trials, presenting new treatment options to improve the conditions of patients with advanced RCC.

III. Innovation and future directions

Innovations in RCC immunotherapies are experimented with through ongoing clinical trials to surpass current limitations and improve patient outcomes. The CheckMate 025 trial, a Phase III study, enrolled patients with advanced or metastatic RCC who had received prior anti-angiogenic therapy (NCT01668784). This trial compared the levels of nivolumab with everolimus, a standard treatment for RCC; the results showed that nivolumab led to an increase in survival and response rates, addressing the limitation of the ability to achieve those standards

with existing therapies. By targeting the PD-1 pathway, nivolumab enhance's the immune system's ability to recognize tumor cells, improving the immune response while offering a new method for patients whose conditions have developed through prior treatments (Deleuze 2020). The CheckMate 214 trial, another Phase III study, enrolled patients with previously untreated advanced or metastatic RCC (NCT02231749). This trial focused on the combination of nivolumab and ipilimumab, an anti-CTLA-4 immune checkpoint inhibitor, compared to sunitinib, a TKI. The combination therapy had much better survival and response rates, especially in patients with intermediate and poor-risk disease. This trial studied the restriction of monotherapy by combining two immune checkpoint inhibitors, improving the anti-tumor immune response and having an effective treatment option for a more versatile range of patients (Considine 2019). These clinical trials document the effect of combining therapies as primary treatments and how significant targeting various immune pathways can be to improve the effectiveness of each method. Current research identifies predictive biomarkers to better select patients who are most likely to benefit from a specific immunotherapy. The use of biomarkers such as PD-L1 expression and tumor mutational burden (TMB) is being experimented to better suit each patient, thus improving treatment outcomes (Rini 2019). Future advancements in RCC immunotherapy also involve the development of agents and combination strategies that target other immune evasion mechanisms, such as the tumor microenvironment and regulatory T cells. Recent methods like personalized cancer vaccines and adoptive cell therapies are being tested to have individualized treatment options (Sheng 2019). The CheckMate 025 and CheckMate 214 trials have significantly advanced RCC immunotherapy approaches by validating the potency of immune checkpoint inhibitors and combination therapies. These trials have focused on previous treatments' key limitations, including high toxicity and restricted efficacy, leading to increased results in ongoing clinical trials.

IV. Conclusion

This study explored the use of immunotherapy in RCC, a type of cancer characterized by the uncontrolled growth of kidney cells. Immunotherapy has shown success in treating RCC by enhancing the immune system's ability to identify and attack cancer cells. Significant types of immunotherapies used for RCC include checkpoint inhibitors like nivolumab and ipilimumab, which have improved patient survival rates. Researchers are currently working to overcome challenges such as immune resistance and identifying biomarkers to optimize treatment options. Looking ahead, immunotherapy has the potential to improve outcomes for RCC patients through more effective treatment methods.

V. References

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11. Clinical Trials
    a. CheckMate 025 trial: NCT01668784 https://clinicaltrials.gov/study/NCT01668784?cond=checkmate%20025&rank=1
    b. CheckMate 214 trial: NCT02231749 https://clinicaltrials.gov/study/NCT02231749