Chapter

Urologic Cancer Molecular Biology

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Abstract

An adequate understanding of the molecular mechanisms of the most common urological cancers is necessary for a correct approach to diagnosis, precise treatment, but also for the follow-up of these patients. It is necessary to understand the molecular mechanisms underlying the carcinogenic processes, the molecular pathways involved in this process, and also to describe the biomarkers useful for diagnosis but also for predictability, treatment, and natural history. In addition, it would be useful to describe a list of useful molecules currently under investigation as possible biomarkers to improve the income of cancer patients.

Keywords: prostate cancer, urothelial cancer, kidney cancer, biomarkers, bioinformatics

1. Introduction

Over the past decades, the treatment of localized cancers was mostly focused on surgery and radiotherapy and advanced neoplasia was treated using nonspecific cytotoxic agents. Despite the increasing 5-year survival rate, there is also still a large number of nonresponsive patients, mostly due to the diversity of genetic profiles among the worldwide population, also the heterogeneity within the tumor itself [1–3].

Neoplasia develops under a various number of molecular and genetic malfunctions that regulate cell division, cell differentiation, and programmed cell death [4, 5]. Tumor suppressor genes and proteins encoded by these genes play a major role in cellular growth regulation, cell signaling, and DNA repair. Oncogenes are mutated forms of normal genes and are associated with cellular proliferation.

Molecular biology focuses on the study of physiological and pathological changes in the body. It helps to develop tools for early diagnosis of these changes and ways to reverse them. In recent years, considerable efforts have been made to elucidate the molecular mechanisms of malignant transformation that have the role of personalized medicine (especially oncology) in order to maximize the effectiveness of the therapeutic response but also to minimize side effects. In this sense, understanding the process of carcinogenesis helps to diagnose at an early stage, an accurate diagnosis but also of the different behavior of tumor subtypes, in order to establish the appropriate therapy [6–11].

BCG (Calmette-Guérin bacillus) immunological therapy in the treatment of bladder cancer is an excellent starting point for the usefulness of molecular studies on immunotherapy in genitourinary cancers. Being a nonspecific agent, there are many gaps regarding its mechanism of action but it paved the way for a different approach, that of inducing an immune response against cancer via cancer vaccines. Prostate and kidney cancer are also considered for this kind of treatment [11].

From a clinical point of view, the most obvious mechanism is the limitation of the specific antigen immune response by CD4 and CD8 (tumor-infiltrating lymphocytes) with significant importance in limiting the antitumor response thus preventing a significant proportion the clinical remission of tumors. Thus, a therapeutic line has been developed that targets an immune checkpoint blockade in order to bypass the mechanisms that limit the response, and which in the case of bladder tumors, in combination with conventional chemotherapy, or VEGF inhibition (vascular endothelial growth factor) in kidney cancer and last but not least, in prostate cancer—hormone therapy, increase the effectiveness of treatment [11].

In this chapter, we discuss the most significant urological cancers including prostate cancer, urothelial carcinoma, and renal highlighting their molecular mechanisms and the related studied biomarkers for precision diagnosis and therapeutic management.

1.1 Bioinformatics in urologic cancers

Cancer is one of the most complex diseases to understand. It is characterized by the rapid growth and spread of its cells, its resistance to conventional treatments, and its ability to invade and displace normal tissue. Malignant cells, regardless of type, usually share some common features—reprogrammed energy metabolism, sustained cell growth signals, evasion of growth suppressors, resistance to apoptosis, facilitation of replicative immortality, induction of angiogenesis, resistance to destruction by the immune system, and promotion of cell invasion and metastasis. These recognized characteristics have led to a deeper understanding of this disease. However, the reality is that our overall ability to cure cancer has not yet improved significantly, especially for adult cancers, which account for 99% of all cancers [12–14].

The major challenges facing clinical oncologists include not only the considerable heterogeneity and different genetic backgrounds even within the same type of cancer, but also the fact that effective drugs lose their efficacy due to the ability of cancer to evolve rapidly, especially with regard to the emergence of drug-resistant subpopulations [12–14].

One of the many reasons why our knowledge is so sparse is the lack of molecular-level data, the full analysis, and interpretation of which can reveal the full complexity of developing cancer. Although large amounts of genomic, epigenomic, transcriptomic, metabolomic, and proteomic data have been obtained for a variety of cancers, few cancer studies are designed to fully exploit all the information that can be derived from the available omic data [12–14]. Integrative analyses of multiple data types may prove to be essential to gain a full and systems-level understanding of cancer's evolution dynamics, including the elucidation of its true drivers as well as key facilitators at different developmental stages of cancer. We anticipate that only when all of the key information hidden in omic data can be fully derived and utilized can we expect a meaningful breakthrough in our understanding of cancer [12–14].

The understanding of the human genome combined with technologies such as DNA and protein arrays or mass spectrometry has improved the simultaneous study

of numerous genes and proteins in single experiments and has rekindled interest in the search for novel biomarkers for cancers such as but not limited to, renal, urothelial, and prostate cancers [15–21]. Modern technology allows for parallel studies as compared to the serial analyses used in older methods. This allows the identification of distinct patterns for cancer diagnosis and classification, as well as for prediction of therapeutic response. In addition, these technologies enable the discovery of new individual tumor markers through the use of acceptable hypotheses and novel analytical methods [15–21]. Although new technologies and tactics often fail in the discovery of established cancer biomarkers and focus on identifying high-incidence compounds, they have the potential to revolutionize biomarker discovery. It is now critical to focus on thorough validation studies to discover the most effective techniques and biomarkers and bring them to the clinic as quickly as possible [15–21].

Bioinformatics and computational techniques have been well applied in the studies of various tumors (urologic, digestive gynecologic, etc.), and confirmed to be efficient and reliable in identifying novel tumor markers for cancer diagnosis and targeted treatments [22].

The very large pool of publicly available cancer-omic data, which includes transcriptomic, genomic, metabolomic, and epigenomic data, contains a considerable amount of information about the activities of individual biochemical pathways, their dynamics, and the complex relationships between them, as well as information about various microenvironmental factors. When the right questions are asked, powerful statistical analysis techniques can be very helpful in uncovering such information. Such targeted questions provide a framework for hypothesis-driven data analysis and evaluation that can be used to test the validity of the formulated hypothesis and to formulate new questions that may lead to the discovery of specific pathways or even possible causal relationships between the activities of different pathways. More effective analysis methods for different omic data formats are definitely needed to answer more difficult and in-depth questions about the data, such as deconvolution of gene expression data obtained from tissue samples with different cell types and inference of causal relationships. Effective data mining and information discovery require integrative analysis of various forms of omic and computational data [14, 15, 17–21].

1.2 Prostate cancer (PCa)

It is considered the second most prevalent cancer among male subjects. Around one in eight men will get diagnosed with the illness during their lifetime. In 2012, around 1.1 million men were diagnosed with prostate cancer globally. Around one in 40 of them will die due to this disease [23–25].

With the discovery and introduction of PSA-based screening tests, the incidence of prostate cancer has increased dramatically. However, given the advances in molecular biology, we realize that a purely PSA-based test does not provide sufficient accuracy. To find an answer, we need to consider other possible screening methods by elucidating the molecular basis of cancer development and more specific biomarkers [23–25].

The complexity of the diagnostic process in prostate cancer is reflected in the various interactions that occur during the course of the disease itself. The initial changes that lead to cancer are usually caused by chronic inflammation and dietary habits. They eventually lead to severe damage to the DNA of the prostate cells. Early genetic events that can promote disease progression include fusions or mutations of various genes and oncogenes (**Figure 1**), as well as malfunctions of molecular signaling pathways [23–25].

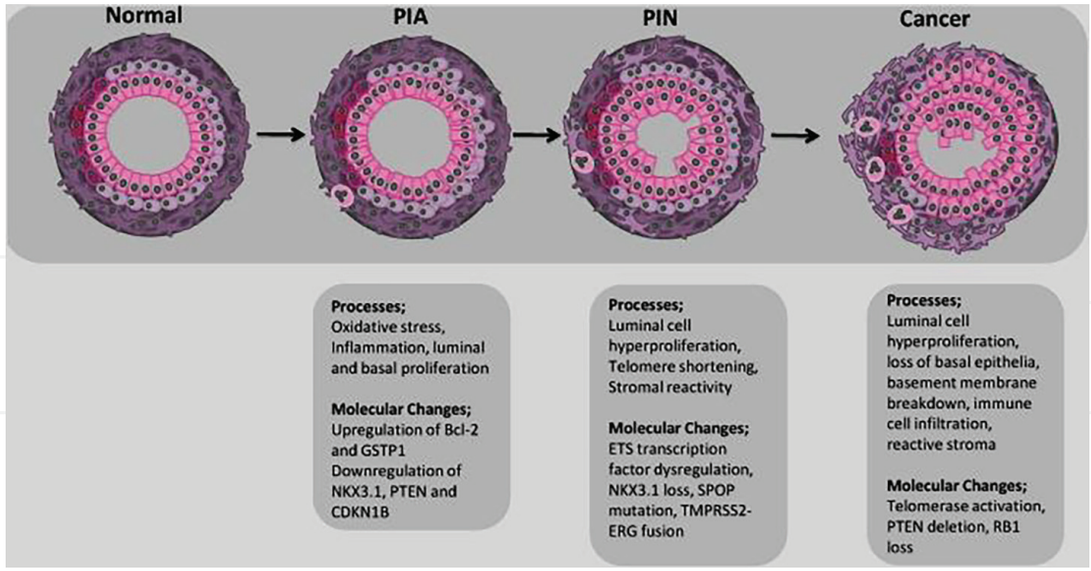

Figure 1.
Alterations that occur in the malignancy of prostatic tissue. PIA - proliferative inflammatory atrophy, PIN - prostatic intraepithelial neoplasia.

The primary androgen of the prostate is dihydrotestosterone (DHT), and exposure to this androgen is considered to be a precipitating factor in the development of primary prostatic neoplasia. The androgen receptor (AR) plays a central role in the development and progression of prostate cancer. Although the relationship between androgen exposure and cancer is not yet clear, exposure to very high or low concentrations of this substance may be protective against PCa (prostate cancer). The effects of androgen on long-term male survival are still unknown. The interaction of vitamin D with its receptor may influence the aggressiveness of the disease and its risk factors, but the mechanism underlying this event is not yet fully understood [23–25].

The prostate develops just caudal to the bladder neck by the proliferation of epithelial buds growing from the urogenital sinus epithelium. Epithelial budding is strictly androgen-dependent and represents the first identifiable events in prostate development. Budding of the prostate requires complicated epithelial-mesenchymal interactions [26, 27].

High testosterone levels in male embryos promote prostate development. Testosterone is converted to DHT by 5α-reductase, an interaction that activates AR. High testosterone levels during early development led to prostate growth regardless of genetic sex, suggesting a primary role for androgens in prostate induction [26, 27].

The upregulation of Sox9 (sex-determining region Y-box 9), a transcription factor induced by the FGF pathway, is the earliest event that appears to occur in the epithelium during prostate development. This mechanism is followed by the increased expression of Nkx3.1 (NK homeobox transcription family member), which influences the degree of branching in the mature mouse prostate, where it may also act as a tumor suppressor [26, 27].

The FGF family of secreted peptides promotes cell growth by binding to cell surface proteins and activating multiple signaling cascades demonstrated for prostate, mammary and salivary glands, or lung. Fgf-7 (keratinocyte growth factor) and Fgf-10 are considered specific for the prostate [28, 29]. Fgf-7 (keratinocyte growth factor) and Fgf-10 are considered specific for the prostate. FGFR2 is expressed in developing prostatic epithelial cells (PrECs) and through interaction with Frs-2α. Both molecules are secreted by the mesenchyme of the prostate. This mechanism led to the hypothesis

that they act as andromedins since they are associated with androgen-independent growth factors. Due to the absence of Fgf-10, the mice also showed prostatic hypoplasia [28, 29].

Wnt signaling plays a crucial role in the development of various organs, including the prostate. Essentially, it regulates proliferation and differentiation through a series of Wnt ligands expressed during prostate bud formation. Canonical Wnt targets, such as Lef1 and Axin2, are upregulated in prostate bud epithelium [30–32].

Prostate cancer is one of the few malignancies for which there is a clinically meaningful serum biomarker. From its discovery in 1979 to its clinical application in the late 1980s to 1990s, PSA has become an invaluable tool for detecting, grading, and monitoring prostate cancer in men.

There is also considerable overlap in serum PSA levels between men with cancer and those with the noncancerous disease. The presence of prostatic hyperplasia or inflammation may also explain the elevated serum PSA levels [33, 34]. To this end, the use of PSA derivatives such as PSA density, PSA velocity, age-adjusted values, and more recently molecular derivatives can be used to improve clinical decisions compared to the isolated use of PSA.

Several molecular approaches have been pursued in the search for the optimal biomarker for prostate cancer. An overview of basic cellular processes begins with a DNA sequence (gene) that is transcribed into mRNA (transcript) and then translated into a protein that can then perform a specific cellular function. A major goal of biomarker development is to identify the differences in the molecular structure of prostate cancer cells compared to their benign counterparts and also to distinguish the more aggressive phenotypes from the others. The identification and quantification of these molecular differences in tissues and body fluids form the basis for the discovery of biomarkers for prostate cancer.

PSMA (glycoprotein prostate-specific membrane antigen), a folate hydrolase, has been studied as a potential biomarker for prostate cancer in tissue, serum, or urine. It is found in the membrane of all prostate epithelial cells. It is a type II transmembrane protein with an extracellular C-terminus that exists as a dimer and binds glutamate and glutamate-like structures [35, 36]. Nowadays, PSMA is mainly used in targeted imaging and theranostics [37, 38]. In particular, 68Gallium positron emission tomography of prostate-specific membrane antigen (68Ga-PSMA PET) is increasingly used as a diagnostic tool in biochemical recurrence after primary therapy [39].

Human kallikrein peptidase 2 (hK2) shares many important properties with PSA and has demonstrated its potential as another tumor marker for prostate cancer. Among many other similarities, hK2 and PSA share 80% amino acid homology, show similar specificity for prostate tissue, and are hormonally regulated by androgens. One of the major functions of hK2 is to activate the zymogen (proPSA) to active PSA by cleaving the amino acid presequence. Critical to its utility as a biomarker is that hK2 expression varies independently of tissue and serum PSA expression. In BPH, PSA expression is highly expressed compared to the minimal immunoreactivity of hK2, but hK2 is also overexpressed in PCa compared to PSA. Furthermore, tissue expression of hK2 appears to correlate with more aggressive pathological features, including Gleason grade [40, 41].

Circulating tumor cells (CTCs) have long been touted as potential prognostic biomarkers and indicators of treatment response. Subsequent CTC research in prostate cancer has employed a wide range of methods utilizing characteristics such as size, surface marker expression, and cellular plasticity that distinguish CTCs from circulating blood mononuclear cells [42, 43]. Typically, CTCs are defined as

CD45 and positive for an epithelial marker such as epithelial cell adhesion molecule (EpCAM) and/or cytokeratin. Although the development of CTCs as biomarkers for prostate cancer has been relatively slow, there has been considerable recent progress in the field and a growing number of clinical trials. Currently, there is only one FDA-approved method for identifying CTCs: CellSearch, which uses antibodies to EpCAM for CTC detection and then stains with antibodies to CD45 and cytokeratins 8, 18, and 19 (positive) to identify individual CTCs. Using this system, a CTC count of five or more cells per 7.5 mL of blood at any time during disease progression has been associated with poor prognosis in the prostate, breast, and colorectal cancers [42, 43].

The ease of collection of urine and the excretion of prostate cells have long made it a potential biomarker source for the early detection of prostate cancer [44]. However, only recently have urine biomarkers for prostate cancer come into clinical use. The first of these biomarkers, described in 1999, prostate cancer antigen 3 (PCA3), is not expressed outside the prostate. Studies show that PCA3 levels in prostate cancer are far higher than those in BPH, but the function of the antigen is still unknown [45, 46]. Recent studies used RT-PCR to detect PCA3 in urine and showed that PCA3 performs better than PSA in diagnosing PCa [47, 48].

Annexin A3 is a protein that is being studied as a possible biomarker for prostate cancer in urine. It belongs to a family of proteins known as phospholipid-binding proteins and shows altered expression in PCa [49].

α-Methylacyl coenzyme A racemase (AMACR) is an enzyme responsible for beta-oxidation of branched-chain fatty acids found in a diet consisting of beef and dairy products. Recent studies have shown that 88% of prostate carcinomas, as well as untreated metastases and hormone-refractory PCa, overexpress AMACR [50]. Immunohistochemical studies have shown that expression of AMACR in prostate tissue has a sensitivity of 97% and a specificity of 100% for the detection of PCa. In conjunction with other markers such as the tumor protein p63, which helps to identify basal cells that are absent in prostate cancer, measuring the expression level of AMACR also can be used for the detection of prostate cancer [51].

Detection at an early stage not only improves the outcome but also reduces mortality in PCa. Although the discovery and use of PSA have revolutionized current PCa detection and treatment, it is not enough. Due to this stage, various molecular modifications or genetic alterations have overtaken the current maximum use of this tumor marker. The use of different PSA derivatives, the discovery of molecular derivatives of PSA, new kallikrein markers, PCA3, and gene rearrangements are leading to a significant improvement in the efficiency of PCa management [24, 25].

1.3 Urothelial cancer (UCa)

The urothelium extends from the renal pelvis to the urethra of the prostate. Urothelial carcinomas (UCa) represent the vast majority of cancers arising in the bladder, and approximately 75% of them are noninvasive within the muscular layer. However, despite the treatment options, there is a high rate of recurrence and, in high-grade tumors, progression to muscle-invasive disease (**Figure 2**). The incidence of UCa increases with age. Most of them are diagnosed in patients over 65 years of age, and it is four times higher in men than in women. One of the reasons for this could be tobacco use, which is known to be a risk factor and is most common in men, although other factors such as the androgen receptor could also play a role [52, 53].

UCa can present a noninvasive phenotype, in which the malignant cells are confined to the urothelial layer, and an invasive phenotype, in which the tumor cells can

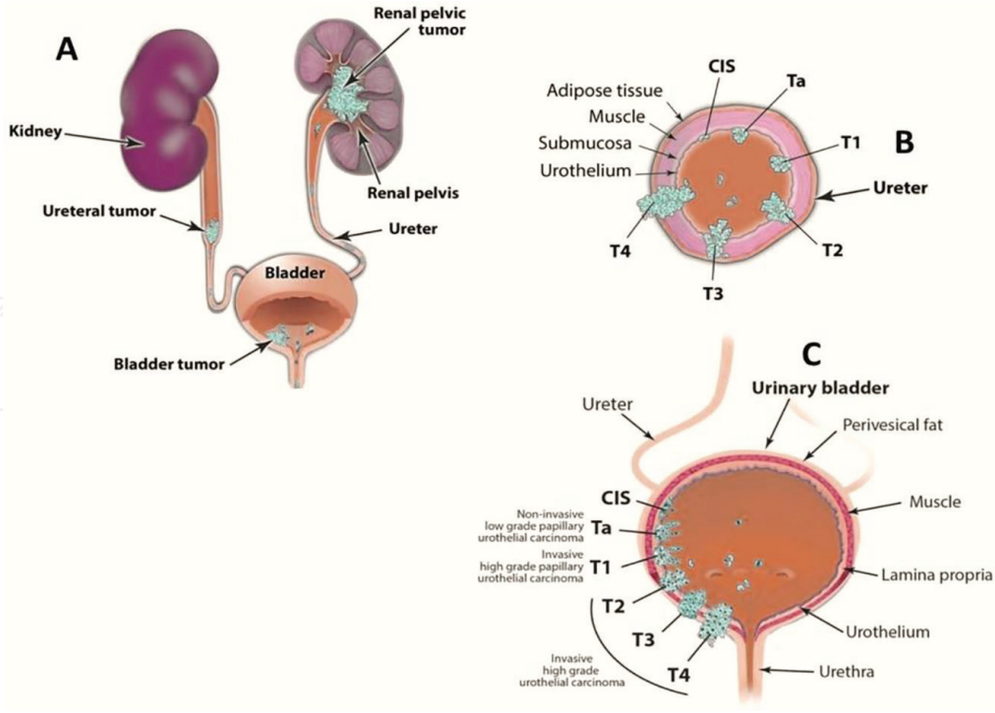

Figure 2.
UCa localization.

break through the basement membrane and invade the subepithelial connective tissue and underlying muscle. There are two types of noninvasive UCa. Exophytic papillary (Ta) tumors are prone to local recurrence but rarely break through the basement membrane or spread. CIS, on the other hand, is a flat lesion with a high susceptibility to invasion and metastasis. Patients who have only CIS lesions in their urinary system are more likely to develop synchronous and/or metachronous malignancies [54]. Ta tumors are caused by molecular abnormalities that are usually separate from CIS and invasive carcinomas, despite the fact that these pathways are not mutually exclusive [55]. The receptor tyrosine kinase-Ras pathway is frequently constitutively active in low-grade papillary carcinomas, with activating mutations in HRAS and FGFR3 [56]. Homozygous deletion of p16INK4a is a common feature in high-grade Ta tumors [57]. TP53 and retinoblastoma (RB) genes and pathways are commonly altered in CIS and invasive malignancies [58]. Although chromosomal-9 deletions can be observed in both dysplastic urothelium and CIS lesions, loss of chromosome 9q heterozygosity is more common in low-grade Ta tumors [59]. When a papillary tumor develops into an invasive phenotype, it is mainly due to the accumulation of additional mutations in the p53 pathway. Invasive cancers have also been shown to have p16 mutations. Matrix metalloproteinases (MMPs), cadherins, TSP-1 (thrombospondin-1), and vascular endothelial growth factors (VEGFs), mutations that alter the extracellular matrix and induce tumor angiogenesis are more common in muscle-invasive cancers and also play a role in nodal metastasis [60].

The most intensively studied aspects of UCa are changes in signaling pathways that affect cell cycle progression. The p53 and Rb signaling pathways, which interact with apoptosis and intracellular signaling mediators, are primarily responsible for cell cycle control. The tumor suppressor gene TP53 is located on chromosome 17p13.1 and encodes the p53 protein. By activating p21WAF1/CIP1, the protein blocks cell cycle progression at the G1-S transition. Inactivation of TP53 and loss of its tumor-suppressive activity may be caused by mutations in the 17p allele [61, 62]. In invasive UCa, loss of

heterozygosity on chromosome 17 is associated with aggressive behavior. Mutations in the TP53 gene result in a protein that is resistant to ubiquitin-mediated degradation. Immunohistochemistry can detect increased intranuclear p53 accumulation as a consequence [63]. Multiple retrospective studies have found that accumulation of p53 in the nucleus is associated with poor prognosis in patients with UCa, particularly in those who have undergone radical cystectomy. From normal urothelium to superficial UCa, muscle-invasive cancer and metastatic lymph nodes, altered p53 expression has been observed to occur [64, 65]. Despite this evidence, the predictive function of p53 in the development and progression of bladder cancer is still debated, but what is certain is that a link between the accumulation of p53 in the nucleus and TP53 mutations has been demonstrated [66].

Mdm2 interacts with p53 in an autoregulatory feedback loop that regulates its activity. Increased p53 levels transactivate the promoter of MDM2, causing the translated protein to facilitate the destruction of p53 by the proteasome. MDM2 levels decrease when p53 levels decrease. In UCa, MDM2 amplification has been found to increase in frequency with tumor stage and grade [67, 68]. p14 inhibits the transcription of MDM2. p14ARF, one of two splice variants derived from the CDKN2A locus on chromosome 9p21, encodes the protein. Because the E2F transcription factor induces p14ARF, it serves as a link between the Rb and p53 pathways. The E2F transcription factor is sequestered by dephosphorylated Rb. E2F is produced when Rb is phosphorylated by cyclin-dependent kinases, leading to the transcription of genes important for DNA synthesis [69, 70].

In UCa, a decrease in Rb protein expression has been highlighted. Rb has been shown to be a predictive factor when combined with other cell cycle regulatory proteins. Cyclin/cyclin-dependent kinase complexes help phosphorylate Rb. CDKIs such as p21, p16, and p27, which act as tumor suppressors, cause negative control of cyclin-dependent kinases. Low levels of p27 have been associated with advanced-stage bladder adenocarcinomas [69–71]. In bladder UCa, p27 mutations have also been associated with poor disease-free and overall survival. In UCa patients treated with radical cystectomy, a combined assessment of p53, p21, Rb, cyclin E1, and p27 has been shown to improve accuracy against each individual molecular marker, thereby improving risk stratification [69–71].

Apoptosis is a tightly controlled process involving a series of events that occur during normal development and in response to a series of stimuli, all leading to programmed cell death. Apoptosis can be triggered in two ways. The internal process is mediated by mitochondria, while the extrinsic system involves the activation of death receptors on the cell surface. Both pathways activate caspases that cleave cellular substrates and allow apoptosis. In urothelial carcinoma cell lines, tumor-specific expression of caspase-8 has been shown to induce apoptosis *in vitro* [71].

The Bcl-2 protein family comprises both antiapoptotic and proapoptotic members, such as Bcl-2, Bax, and Bad, and is involved in the intrinsic apoptotic process. In UCa patients treated with radiotherapy or synchronous chemoradiotherapy, increased Bcl-2 expression has been associated with poor outcomes. In patients with advanced UCa undergoing radiotherapy who might benefit from neoadjuvant chemotherapy, Bcl-2 could serve as a marker [72, 73]. Expression of Bcl-2 has been associated with a lower tumor-free survival rate in high-grade T1 tumors, and in combination with p53, it may be a strong prognostic indication in non-muscle-invasive UCa. In addition, a prognostic index based on Mdm2, p53, and Bcl-2 was developed, with abnormalities in all three markers corresponding to the lowest probability of survival in UCa [74, 75]. Bax expression, on the other hand, is an independent predictor of better prognosis

in invasive UCa. The proapoptotic function of Bax is mediated by the activation of Apaf-1. In UCa patients, lower Apaf-1 expression has been associated with a higher mortality rate [76, 77].

Multiple cell-surface receptors modify signals from the environment and transmit them to the nucleus of urothelial cells via transduction pathways. Uncontrolled cellular proliferation and tumor growth may result from alterations in these receptors and/or the signals sent. Activating mutations of FGFR3 are the best-studied alterations in UCa in the FGFR family. FGFR3 mutations are found in nearly 60–70% of low-grade papillary Ta tumors [78].

ErbB-1 and ErbB-2 (Her2/neu), members of the epidermal growth factor receptor (EGFR) family, are overexpressed in invasive UCa. Overexpression of ErbB-1 has been associated with an increased risk of progression and mortality [79]. Increased ErbB-2 expression has also been associated with aggressive UCa as well as poor disease-specific survival. In contrast, other studies have found that ErbB-2 expression is not related to prognosis. While it has been suggested that the combined expression profile of ErbB-1 and ErbB-2 is a stronger predictor of prognosis than either marker alone, this finding remains to be confirmed [80–82].

JAK (Janus kinase) is a tyrosine kinase that is activated by cytokines and growth receptors and regulates a variety of signaling pathways. JAK signaling is thought to be increased by overexpressed preoperative plasma levels of interleukin-6, a ligand for the corresponding cytokine receptor, and is an independent predictor of UCa recurrence and survival [83]. The activation of the STAT (signal transducer and activator of transcription) pathway, which controls transcription of several key genes, is the most studied molecular event after JAK activation. STAT1 inhibits Bcl-2 expression, whereas STAT3 has the reverse effect. In UCa patients, STAT3 expression in combination with other markers can predict the likelihood of recurrence and survival [84].

Angiogenesis is the process of cancer cells producing substances that interact with stromal components to recruit endothelial cells to the site of cancer and generate a vascular supply that gives cancer cells with the nutrients they need to proliferate [85, 86].

VEGFs are signaling proteins that stimulate angiogenesis by interacting with VEGF receptors and stimulating cellular responses (VEGFRs). The majority of known cellular responses to VEGF are mediated by VEGFR2. Advanced UCa and muscle invasion are linked to VEGFR2 expression. In UCa patients, VEGFR2 expression is also a key determinant of nodal metastasis. VEGF boosts nitric oxide synthase, which boosts nitric oxide production and tumor vascularization. In nonmuscle-invasive UCa, VEGF overexpression is linked to early recurrence and progression [87, 88]. VEGFs are signaling molecules that promote angiogenesis by interacting with VEGF receptors and stimulating cellular responses (VEGFRs). The majority of known cellular responses to VEGF are mediated by VEGFR2. Advanced UCa and muscle invasion are linked to VEGFR2 expression. In UCa patients, VEGFR2 expression is also the main predictor of nodal metastasis. VEGF boosts nitric oxide synthase, which boosts nitric oxide production and tumor vascularization. In nonmuscle-invasive UCa, VEGF overexpression is linked to early recurrence and progression [89, 90].

The ability of urothelial cancer cells to invade blood vessels and lymphatics is essential to their ability to spread to nearby structures and form distant metastases. Cadherins are intercellular adhesion mediators that have been identified in a variety of tissues. E-cadherin is the most known member of the cadherin family and is essential for epithelial cell adhesion. In UCa, lower E-cadherin expression has been linked to an increased risk of tumor recurrence and progression and shorter survival [91, 92]. The action of various protease families, including uPAs and MMPs, enhances

the ability of a tumor to degrade the matrix and infiltrate the basement membrane. Thymidine phosphorylase (TYMP), an enzyme that increases MMP synthesis, is overexpressed in advanced UCa compared with superficial tumors or normal bladder tissue [93]. Increased thymidine phosphorylase nuclear reactivity has been associated with an increased prevalence of superficial UCa recurrence. MMP-2 and MMP-9 expression levels have been shown to be associated with the stage and grade of urothelial tumors. Increased MMP-2 expression may also indicate a poor prognosis for recurrence-free and disease-specific survival. In UCa patients, the ratio between MMP-9 and E-cadherin is a predictive factor for disease-specific survival [94, 95].

Integrins are transmembrane glycoproteins that can promote tumor development, invasion, and metastasis when their function is disrupted. They are protein receptors for adhesion molecules and collagen. The immunoglobulin superfamily member intercellular adhesion molecule 1 (ICAM1) interacts with particular integrin classes. ICAM1 expression is linked to an infiltrative histological phenotype, according to immunohistochemical investigations. The presence, grade, and size of bladder tumors have all been linked to serum ICAM1 levels [96].

In patients with UCa of the bladder, ICAM1 is part of a multimarker model that can predict nodal status. The α6β4 integrin is tightly connected to collagen VII in normal urothelial cells and inhibits cell migration. Superficial UCa has shown loss of polarity of α6β4 expression, and invasive tumors reveal either loss of α6β4 and/or collagen VII expression or lack of colocalization of either protein. Patients who have malignancies with weak α6β4 immunoreactivity have a better prognosis than those who have tumors with no or significant overexpression. Overall, molecular invasion indicators are relatively accurate predictors of outcome in UCa patients [97].

Circulating tumor cells are the most basic blood-based biomarker. The presence of tumor cells in the blood has been associated with advanced disease stages in various solid organ cancers. In a recent study, the predictive value of the amount of circulating tumor cells obtained with CellSearch technology was investigated in 100 UCa patients who had undergone cystectomy. About 25% of clinically localized Uca patients had circulating tumor cells, the researchers found, and they associated the results with a worse outcome for these patients [98].

Ki-67 is a nuclear protein that is synthesized by proliferating cells that is used to determine the percentage of cell growth fraction. In patients with superficial UCa, the cell proliferative index is associated with prognosis, and the Ki-67 antigen is a strong predictor of progression, recurrence, and treatment response. This result was confirmed in patients receiving cystectomy who had muscle-invasive UCa [99, 100].

Survivin is also an apoptosis inhibitor that can bind caspases after their activation and prevent them from cleaving their substrates. Survivin expression has been shown to be associated with bladder cancer progression and mortality, and its function as a prognostic indicator has been externally validated. In a multiplex panel including other apoptosis-regulating genes, survivin has been shown to predict tumor recurrence after cystectomy and mortality more accurately than clinicopathological factors alone [101].

COX-2 is an enzyme known primarily for being a target for nonsteroidal anti-inflammatory drugs. Increased levels of COX-2 have been studied in both the upper and lower urinary tract as a marker of UCa angiogenesis and tumor aggressiveness. COX-2 was increased not only in upper urinary tract carcinomas but also in nearby nontumor cells (stromal cells), suggesting an association between more aggressive upper urinary tract malignancies and worse prognosis [102, 103].

IGF (insulin growth factor) and IGFBP-3 (insulin growth factor binding protein-3) are circulating proteins that function as growth signal mediators and

mitogens, respectively. IGF and IGFBP-3 levels were measured preoperatively in individuals having cystectomy to see if they may be used as blood-based predictors of UCa outcome. Although individual marker levels were not efficient, an association between the two of them (low IGF-adjusted IGFBP-3 levels) was a predictor of distant metastases and poor survival [104].

Periplakin is a protein that is found in normal cellular desmosomes and is encoded by the PPL gene. In a cohort study of UCa patients, serum circulating periplakin was investigated and compared to 30 healthy subjects. While UCa patients had considerably lower serum periplakin levels than controls, this difference was diminished in patients with invasive tumors [105].

Bladder cancer is being more recognized as a disease that cannot be treated merely based on pathologic staging; instead, therapeutic efforts must focus on molecular abnormalities in particular tumors. The formation and course of urothelial malignancies have been better understood, thanks to the availability of advanced molecular profiling and computational methods. Future Uca treatment will rely on consensus marker panels to provide accurate prognosis and therapeutic response predictions in individual patients. The disease will be effectively treated if patients are stratified based on risk factors and tumor expression signatures, followed by optimum surgical treatment and disruption of important signaling pathways through the use of therapies targeting several molecular pathways [106].

1.4 Kidney cancer

Kidney cancer is the fourteenth most prevalent cancer in the world, with men having the ninth most common case and women having the fourteenth most common incidence [107, 108].

Renal cancers in adults include malignant tumors of the renal parenchyma and pelvis, but benign tumors and inflammatory causes should also be considered in the differential diagnosis of a renal mass. The majority of tumors arising from the renal pelvis are urothelial tumors, which account for less than 10% of all renal carcinomas. Renal cell carcinoma (RCC), also known as renal adenocarcinoma, is far more common than benign tumors or other malignancies and accounts for 90% of all kidney cancers. RCC can be divided into several histological subgroups, each with its own clinical features and evolution [109].

The clear cell type of renal cell carcinoma is the most common, accounting for 75% of new cases, followed by the papillary, chromophobe, medullary, and collecting duct subtypes, which account for 10%, 5%, 1%, and 1% of new cases, respectively [107].

Von Hippel and Lindau characterized a vascularized developmental pattern of the retina that was later identified as part of an autosomal dominant disease. Hemangioblastomas, pheochromocytomas, and clear cell renal carcinomas were also common in these patients. Up to 90% of sporadic RCCs have somatic mutations, promoter methylation, or loss of heterozygosity of VHL. The VHL protein is known to function as a substrate recognition component of an E3 ligase and for ubiquitination and degradation of HIF (hypoxia-inducible factors) [110–112].

The alpha subunit of HIF heterodimerizes with HIFβ under hypoxic conditions or in the absence/inactivation of the VHL protein, translocates to the nucleus, and transcribes a variety of genes, including VEGF, PDGF, and TGF. In most spontaneous RCCs, inappropriate activation of this system is a major cause of angiogenesis, invasion, and metastasis [113].

In metastatic or unresectable RCC, targeting the VEGF pathway has been a cornerstone of treatment. In metastatic RCC, small molecule TKI (tyrosine kinase inhibitors) have proven successful in interrupting VEGF signaling, resulting in longer patient survival. Endothelial cells can be stimulated to proliferate and migrate by VEGF and PDGF [113].

The development of an increased blood supply can promote the development of metastatic niches and lead to the spread of the tumor. Because of this significant metastatic potential, no neoadjuvant systemic treatment is currently accepted for RCC with targeted therapies such as sunitinib or pazopanib. These agents are also not approved for adjuvant treatment after nephrectomy. Several studies have failed to demonstrate that adjuvant TKIs or immunotherapies improve survival after definitive surgery, underscoring the need for early intervention with surgery upfront. The most common genetic mutation in RCC is the loss of chromosome 3p. This region contains the PBRM1 gene in addition to VHL (3p21) [114].

PBRM1 is a "gatekeeper" gene that helps in DNA repair, replication, and transcription. Somatic mutations have been detected in 41% of clear cell renal carcinomas, with estimates ranging from 40 to 50%. Loss of PBRM1 is associated with advanced disease stages, a higher grade cancer, and poorer treatment outcomes [115–117].

Mutations on chromosome 3p could indicate an important genetic event, whether inherited or acquired, that drives early carcinogenesis. RCC features a number of genomic changes, including an increase in 5q with TGFB1 and CSF1R, as well as a 14q deletion with the tumor suppressor candidate NRXN3. Loss of 14q has been related to a progression of the disease and a reduced life expectancy [118, 119].

mTOR is a serine/threonine kinase that forms two different complexes with adaptor proteins—mTORC1 and mTORC2. mTORC1 activity was found in more than half of all RCCs. HIF-1α has been demonstrated to promote the expression of REDD1, a proven mTORC1 inhibitor. Stabilization of HIF1 levels under hypoxic environments causes mTOR signaling to be inhibited [120].

Mutations in TSC1 and PTEN may prevent the HIF-1 signaling axis from inhibiting mTOR, resulting in a second, independent mechanism of carcinogenesis. Everolimus as shown in **Figure 3**, suppresses the activity of mTORC1 via binding to FKBP-12 [121, 122].

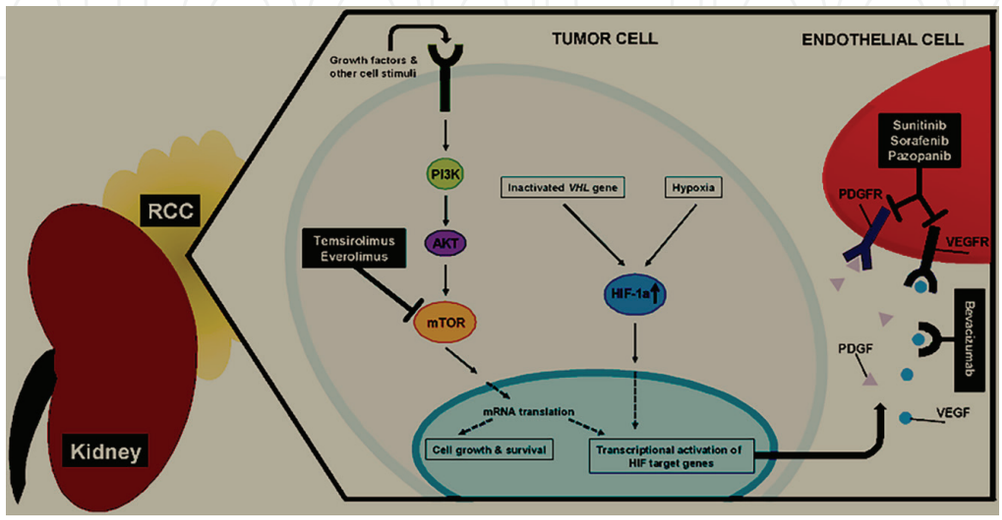

Figure 3.
Current therapeutic management of RCC.

Over the past two decades, research has focused on molecular events that can uncover the biological heterogeneity underlying the diverse clinical behavior of RCC, with the expectation of identifying accurate markers that can personalize prognosis and risk-stratified clinical management, as well as predict response to existing therapeutic approaches [123].

Molecular biomarkers are associated with clinical and/or pathologic characteristics of RCC and have an effect on progression-free survival, OS, cancer-specific mortality, and prognosis [124].

In addition, new research has recently been published on PBRM1 (polybromo 1), BAP1 (BRCA1-associated protein 1), and SETD2 (SET domain-containing protein 2). Although these biomarkers are targeted by a variety of RCC treatments, their prognostic and predictive value has yet to be confirmed internally and externally. All tyrosine kinase inhibitors, such as bevacizumab, target VEGF, while some others, such as cabozantinib, target a larger variety of receptors, including AXL and the protooncogene c-met [123].

As explained earlier, VHL is responsible for the degradation of HIF-α. As a result, changes in VHL proteins lead to HIF-α accumulation in addition to hypoxic cell conditions. HIF-α is a key player in cancer pathogenesis, activating approximately 30 genes involved in tumor proliferation and angiogenesis, including the overexpression of VEGF. When ccRCC (clear cell renal cancer) is compared with papillary or chromophobe forms of RCC, HIF-α expression is significantly higher. In both clear cell and papillary RCC, studies have reported no difference in survival between patients with low or high HIF-α expression, while other studies have found a worse prognosis with increased HIF-α in cancer cells [123].

VEGF is a dimeric glycoprotein that promotes tumor growth and metastasis by influencing angiogenesis in both normal and pathological situations. Due to HIF-α dysregulation and hypoxia caused by an inadequate blood supply in larger tumors, VEGF production is increased in ccRCC. RCC patients with VHL gene mutations and advanced tumor grade have higher VEGF levels and secretion. VEGF expression correlates with tumor necrosis, microvessel invasion, tumor stage, and Fuhrman grade in ccRCC, in addition to tumor grade and size. In studies, increased VEGF levels have been observed to decrease progression-free and overall survival rates of RCC [123, 125].

The additional value of VEGF, despite its promising properties, has yet to be confirmed and externally validated. C-met is a receptor tyrosine kinase and a protooncogene. Angiogenesis, tissue regeneration, cell proliferation, and differentiation are controlled by this protein. Mutations in the c-met signaling pathways have been linked to a variety of tumors, including all forms of RCC [123, 125]. The upregulation of c-met has been linked to the VHL mutation in ccRCC. It has been found that c-met expression is particularly high in tumors with papillary and sarcomatoid differentiation. In recent studies, increased c-met expression was found to reduce cancer-specific mortality. Further research is required to fully understand the role of c-met in the etiology of RCC [126, 127].

Transmembrane protein CAIX is linked to tumor development, poor prognosis, and aggressive phenotype. CAIX is thought to be involved in the regulation of the tumor microenvironment, particularly the fluctuations in intracellular and extracellular pH in response to hypoxia in the tumor, and is regulated by HIF. CAIX is expressed in more than 80% of RCC samples and 90% of ccRCC samples and can therefore be used to confirm the diagnosis of RCC. CAIX expression has been associated with better prognosis and survival in patients with localized RCC and mRCC,

and with an inverse relationship with metastatic spread. In contrast, low CAIX expression was not associated with renal cancer mortality [128].

CAIX may be more useful in identifying small renal tumors. With the advancement of technology, three genes have been discovered to be altered in more than 10% of sporadic clear cell RCC: BAP1, PBRM1, and SETD2. We can assume that these genes play an important role in renal cell carcinoma because, as in VHL, they are tumor suppressor genes with two hits found on the short arm of chromosome 3p [129].

The mTOR pathway regulates cell proliferation, protein degradation, and angiogenesis as part of the biological response to environmental stress. PTEN (phosphatase and tensin homolog) is an upstream molecule in this process, while phosphorylated S6 ribosomal protein is a downstream molecule. The use of temsirolimus, an mTOR inhibitor, as a first-line treatment for low-risk patients is recommended in recent treatment guidelines. In addition, recent studies have shown that altering regulators of the mTOR pathway improves the accuracy of prognostic models as well as the ability to predict recurrence in ccRCC patients who had undergone nephrectomy [130, 131].

pS6 (ribosomal protein S6) is a downstream mTOR target that has been associated with the activation of the mTOR pathway. Due to phosphorylated pS6 activity, it exhibits S6 kinase activity that affects mRNA translation. PS6 is overexpressed in clear cell mRCC and could be used to predict survival in both localized and nonlocalized mRCC. pAkt (protein kinase B) regulates both growth and survival mechanisms by phosphorylating substrates in the cytoplasm and nucleus. Elevated pAkt is associated with lower RCC-specific survival, higher grade, and faster progression of metastasis [123, 132]. Overexpressed pAkt, on the other hand, was linked to a better prognosis in localized RCC. According to recent studies, the localization of pAkt may be essential in defining tumor behavior and thus prognostic value. They discovered a higher level of nuclear pAkt in localized RCC tissue than in mRCC tissue [123, 133].

The tumor suppressor protein PTEN is encoded by the tumor suppressor gene PTEN and is located upstream of mTOR. Via PI3K, PTEN inhibits the phosphorylation of pAkt. PTEN mutation is uncommon in renal cancer and is linked to a high mortality rate. PTEN expression is observed in cancers with a lower T stage and a nonclear cell histological subtype and increases survival [123, 133].

CAF (cytokine and angiogenic factors), survivin, caveolin-1, p53, vimentin, insulin-like growth factor II mRNA-binding protein 3, matrix metalloproteinases, fascin, ki-67, tumor necrosis, and c-reactive protein are examples of other biomarkers. Survivin is a member of the family of apoptosis inhibitors that are active in both the intrinsic and extrinsic caspase pathways [132]. It regulates mitotic progression and promotes alterations in gene expression associated with tumor cell invasiveness. Survivin mRNA is typically expressed during embryonic and fetal development and then disappears in most differentiated adult tissues. Survivin is overexpressed in a variety of malignancies, including all forms of RCC [123].

Given the importance of deregulation of apoptosis in carcinogenesis, it is not surprising that high expression of survivin is associated with poor differentiation, aggressiveness, and lower survival in ccRCC. The p53 protein is a DNA-binding molecule involved in transcription and the regulation of cell growth. When DNA damage occurs, p53 initiates apoptosis and causes cell cycle arrest. Overexpression of p53 has been found in all forms of RCC, especially papillary RCC. Although p53 has been shown to be an independent predictor of metastasis-free survival in patients with localized clear cell RCC, its prognostic significance in RCC is still debated [123].

MMPs are overexpressed in all forms of RCC cancer, especially in nonclear cell RCC tumors, and are associated with aggressive behavior, tumor grade, and survival. Batimastat (synthetic) and bryostatins (natural) are MMP inhibitors that may help to treat and prevent MMP-overexpressing malignancies [123].

IMP3 (insulin-like growth factor II mRNA-binding protein 3) is an RNA-binding protein found in oncofetal tissues. Insulin-like growth factor II mRNA transcription is regulated by it. IMP3 is expressed during embryogenesis in a variety of developing tissues, including epithelium, muscle, and the placenta. In adult tissues, however, it is expressed at low or undetectable levels. In several malignancies, including RCC, IMP3 is associated with cell proliferation and invasion. Stage, grade, sarcomatoid differentiation, regional lymph node involvement, distant metastasis, and cancer-specific mortality are all associated with IMP3. The inclusion of IMP3 expression in the tumor stage increases metastatic progression prediction of metastatic progression and the predictive value of IMP3 in ccRCC was externally validated by researchers [123, 134, 135].

Ki-67 is a cell proliferation marker that has been linked to higher recurrence rates, a more aggressive phenotype of ccRCC, and a poor prognosis. The combination of Ki-67 and CAIX improves the predictive power of nuclear grade in assessing cancer-specific mortality. Complementary studies are needed to evaluate its significance as a prognostic factor [123].

Caveolin-1 is a structural component of caveolae, microdomains of the plasma membrane that regulate cell adhesion, growth, and survival through intracellular signaling. Caveolin-1 is detected in 86% of ccRCCs and 5% of chromophobe and papillary RCCs. The caveolin-1 expression has been linked to a poor clinical outcome in a variety of cancers [123].

One of the components of the scoring algorithm of Leibovich et al. is tumor necrosis. The importance of this component in the prognosis of RCC has led to some debate. When typical clinical and/or pathological tumor features were considered, several studies found that tumor necrosis had no additional value. In contrast, Lam et al. found that tumor necrosis improved survival prediction in patients with localized RCC [136, 137].

The inflammatory marker C-reactive protein has been shown to be a significant predictor of metastasis and overall mortality after nephrectomy for localized renal cell carcinoma. It improved the predictive accuracy of a number of known clinical and pathological predictors by up to 10%. Karakiewicz et al. studied and stated CRP as an independent predictor of mortality in RCC [138].

They also discovered that CRP improved the accuracy of the UISS prediction model. According to Michigan et al., elevated CRP was associated with increased mortality in patients undergoing nephrectomy. Erythrocyte sedimentation rate (ESR), another inflammatory marker, was also associated with higher all-cause mortality. Because they are affordable and readily available, these markers are very promising [123, 127].

Vimentin is a cytoplasmic intermediate filament that should not normally be detected in epithelial cells. Its overexpression has been observed in up to 51% of ccRCC and 61% of papillary RCC, and it has been associated with poor outcomes, regardless of T stage or grade [123].

Fascin is a globular actin cross-link protein that plays a role in cell motility and adhesion. Its overexpression has been associated with sarcomatoid tumors, their stage, grade, size, and metastatic ability [123].

CTLA-4 is a protein described on the surface of cytotoxic T lymphocytes. It is thought to reduce inflammation by preventing tumor-infiltrating lymphocytes (TILs) and T cell activation by preventing tumor cell B71 from binding to CD28. The presence of CTLA-4 has been associated with higher tumor grade in RCC. Lymphocytes also have a cell surface receptor, PD-1. It belongs to the immunoglobulin family and binds to the ligands PD-L1 and PD-L2, which are found on almost all cells, including tumor cells. They are thought to promote apoptosis by decreasing the activity of cytotoxic T cells [139]. In addition, tumor cells are thought to express PD-L1/B7-H1 to prevent tissue destruction by an activated immune system [123, 127].

PD-1 inhibitors, particularly nivolumab, have been the subject of numerous studies, all of which have yielded promising results. The FDA approved nivolumab as second-line therapy for RCC in 2015, based on the results of a study that OS showed benefit, good tolerability, and improved health-related quality of life with nivolumab treatment. It is worth noting that ongoing trials using a mix of targeted treatments, such as anti-VEGFs, and nivolumab are showing promising results [122, 123].

Conflict of interest

The authors declare no conflict of interest.

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