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Invited Review

Combination immune checkpoint and targeted protein kinase inhibitors for the treatment of renal cell carcinomas

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ABSTRACT

Kidney cancers comprise about 3% of all new malignancies in the United States. Renal cell carcinomas (RCCs) are the most common type of renal malignancy making up about 85% of kidney cancer cases. Signs and symptoms of renal cell carcinomas can result from local tumor growth, paraneoplastic syndromes, or distant metastases. The classic triad of presentation with flank pain, hematuria, and a palpable abdominal mass occurs in fewer than 10% of patients. Most diagnoses result from incidental imaging findings (ultrasonography or abdominal CT imaging) performed for another reason. Localized disease is treated by partial nephrectomy, total nephrectomy, or ablation (tumor destruction with heat or cold). When the tumors have metastasized, systemic therapy with protein-tyrosine kinase antagonists including sorafenib, sunitinib, pazopanib, and tivozanib that target vascular endothelial, platelet-derived, fibroblast, hepatocyte, and stem cell factor growth factor receptors (VEGFR, PDGFR, FGFR, MET, and Kit) were prescribed after 2005. The monoclonal antibody immune checkpoint inhibitor nivolumab (targeting programed cell death protein 1, PD1) was approved for the treatment of RCCs in 2015. It is usually used now in combination with ipilimumab (targeting CTLA-4) or cabozantinib (a multikinase blocker). Other combination therapies include pembrolizumab (targeting PD1) and axitinib (a VEGFR and PDGFR blocker) or lenvatinib (a multikinase inhibitor). Since the KEYNOTE-426 clinical trial, the use of immune checkpoint inhibitors in combination with protein-tyrosine kinase inhibitors is now the standard of care for most patients with metastatic renal cell carcinomas and monotherapies are used only in those individuals who cannot receive or tolerate immune checkpoint inhibitors.

1. Tumors of the kidney

1.1. Incidence, types, and presenting signs and symptoms

Malignant neoplasms of the kidney, which accounted for about 2.2% of cancers worldwide in 2020, encompass a heterogeneous group of tumors that are usually derived from renal tubular epithelial cells [1]. At that time, there were about 271,000 and 160,000 new cases (a ratio of 1.6:1) and 116,000 and 64,000 deaths in men and women (1.8:1), respectively. Kidney cancers comprise about 3% of all new malignancies in the United States where the estimated number of new cases for 2024 is about 52,000 and 29,000 (1.8:1) with about 9450 and 4940 deaths in men and women (1.9:1), respectively [2]. Renal cell carcinomas (RCCs), which occur in older patients with a median age at the time of diagnosis of about 63 years, are the most common type of renal malignancy making up about 85% of kidney cancer cases [3]. Risk factors for renal cell carcinoma development include cigarette smoking, obesity, hypertension, and chronic kidney disease.

Clear cell RCC accounts for about 75% and non-clear cell RCC accounts for about 20% of renal cell cancers [3]. Clear cell and non-clear cell nomenclature is based upon the histologic appearance of the tumors under hematoxylin and eosin staining. The remaining 5% are

Abbreviations: C-spine, catalytic spine; CS1, catalytic spine residue 1; CL, catalytic loop; C_H, constant heavy chain; C_L, constant light chain; CT, computed tomography; EGFR, epidermal growth factor receptor; FDA, the Food and Drug Administration of the United States; FGFR, fibroblast growth factor receptor; GRL, glycine-rich loop; HR, hazard ratio; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD1, programed cell death protein 1; PDL, programed cell death protein 1 ligand; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PFS, progression-free survival; RCC, renal cell carcinoma; RECIST, Response Evaluation Criteria in Solid Tumors; R-spine, regulatory spine; RS1, regulatory spine residue 1; Sh2, shell residue 2; VEGFR, vascular endothelial growth factor receptor; V_H, variable heavy chain; V_L, variable light chain.

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unclassifiable or other minor tumor subtypes. Clear cell tumors exhibit clear or granular cytoplasm owing to the presence of lipids and glycogen. Non-clear cell RCC includes papillary (10–15%) and chromophobe (5%) carcinomas and several minor types including *TFE3*-rearranged, *TFEB*-altered, and collecting duct (Bellini) types [4]. Papillary carcinomas are composed of cuboidal cells arranged in papillary formation [5]. Chromophobe renal carcinomas consist of pale eosinophilic cells arranged in solid sheets with a perinuclear halo. Collecting duct carcinomas show irregular channels lined by very atypical epithelia. One of the unusual characteristics of RCC is its tendency to invade the renal vein and grow as a solid column of cells that extends up the inferior vena cava, occasionally as far as the right side of the heart.

Inherited or familial syndromes leading to renal cell carcinomas account for about 3% of cases, with von Hippel-Lindau disease being the most prevalent [4]. This disorder is caused by an autosomal-dominant mutation in the *VHL* gene that predisposes to benign and malignant tumors. The *VHL* gene encodes a protein that forms part of a ubiquitin ligase complex that targets, inter alia, the HIF-1α (Hypoxia-Inducible Factor-1α) and HIF-2α transcription factors for degradation. When VHL is inactive, HIF-1α/2α levels remain high causing inappropriate expression of a number of genes including those that promote angiogenesis such as VEGF, VEGF-C, PlGF (placental growth factor), VEGFRs, MET (the hepatocyte growth factor receptor), and FGFRs [3,6].

Signs and symptoms of renal cell carcinomas can result from local tumor growth, paraneoplastic syndromes, or distant metastases [3]. The classic triad of flank pain, hematuria, and a palpable abdominal mass occurs in fewer than 10% of patients. Most diagnoses result from incidental imaging findings (ultrasonography or abdominal CT imaging) performed for another reason. The symptoms produced by paraneoplastic syndromes, which are common in RCCs, result from cytokines or hormones released by tumor cells or by an immune response against the tumor. Signs and symptoms include fever, hypercalcemia (nausea, poor appetite, muscle weakness or twitching, fatigue), and erythrocytosis (hypertension, nose bleeds, headache, blurred vision, red skin on the face, hands, and feet). Most of these symptoms are abated following tumor resection. Imaging studies often aid in the diagnosis of clear cell renal carcinomas. Typical features include outward tumor growth, heterogeneity owing to tumor necrosis or hemorrhaging, and high uptake of contrast-enhancement agents such as gadolinium for MRIs or iodinated contrast for CT imaging.

1.2. Staging of kidney cancers

Tumor staging is based upon size, the magnitude of spread outside of the kidney, lymph node involvement, and the presence of metastases [3]. In stage I RCC, the tumor is less than 7 cm in its largest dimension and is confined to the kidney. In stage II RCC, the tumor is greater than 7 cm and is confined to the kidney. The five-year survivals are about 95% and 88% for these two stages, respectively. Both of these stages are treated by partial nephrectomy, complete (radical) nephrectomy, or ablation (tumor destruction with heat or cold). In stage III RCC, the tumor is found in the major veins or has regional lymph node involvement with an intact fascia renalis (Gerota's fascia). Treatment includes radical nephrectomy, adrenalectomy, and tumor thrombus excision (if applicable), and lymph node excision. The five-year survival rate is about 59%. In stage IV RCC, the tumor has distant metastasis and extends beyond the fasica renalis (a thin membrane sheath enclosing the kidney that is formed by the condensation of the fibroareolar tissue surrounding the kidney and perirenal fat). These patients are treated systemically with immune checkpoint inhibitors, VEGF or VEGFR blockers, antagonists of other signal transduction modules, or a combination of them, as described later.

2. Treatment of renal cell carcinomas with small molecule targeted therapies

2.1. Overview of targeted therapies

Several drugs have been licensed by the FDA for the treatment of metastatic renal cell carcinomas including several VEGF receptor protein-tyrosine kinase inhibitors, a VEGF-A binding monoclonal antibody (bevacizumab), two mTOR inhibitors (everolimus and temsirolimus), and four additional biologics (pembrolizumab, nivolumab, ipilimumab, and avelumab) [3,7]. Bevacizumab is a humanized monoclonal antibody that is FDA-approved for the treatment of colon (2004), lung (2006), and ovarian cancers (2018), glioblastoma (2009), and renal cell carcinomas (2009). Bevacizumab is given intravenously at 14-day intervals. Clinical trials that led to the approval of this biologic are given in Refs. [6,8–10]. Monoclonal antibodies are produced by a B cell hybridoma (a cell line derived by the fusion of a single normal B cell and an immortal B cell tumor line). César Milstein (www.nobelprize.org/uploads/2018/06/milstein-lecture.pdf) and Georges Köhler (a postdoctoral fellow with Milstein) (www.nobelprize.org/uploads/2018/06/kohler-lecture.pdf) received the Nobel Prize in Medicine or Physiology in 1984 for developing the hybridoma methodology for producing monoclonal antibodies.

2.2. Therapeutic small molecule protein kinase antagonists

Sorafenib is a pyridine-urea derivative (Fig. 1A) that was the first small molecule protein kinase inhibitor approved by the FDA (2005) for the treatment of renal cell carcinomas [11]. The compound is a broad-spectrum antagonist with activity against VEGFR1/2/3, B- and C-Raf, Kit (the stem cell factor receptor), Flt3, RET (the receptor for glial-cell derived neurotrophic factors), and PDGFRA/B [12–18]. A placebo-controlled phase III study was performed in RCC patients, which randomized a total of 903 participants, most of whom were previously treated [19–21]. The objective response rate (ORR) was 10% for sorafenib and 2% for the placebo. The objective response rate is the percentage of subjects in a study group who have a partial or complete response to the treatment within a certain timeframe. A partial response signifies a decrease in the size of a tumor or in the amount of cancer in the body, while a complete response is the disappearance of all signs of the cancer in the body. The objective response rate is a common endpoint used in clinical trials of cancer drugs for solid tumors. Stable disease was observed in 78% and 55% of patients on sorafenib and the placebo, respectively. Sorafenib significantly prolonged median progression-free survival (PFS) to 5.5 months compared with 2.8 months for the placebo in all subsets of patients evaluated (Hazard Ratio or HR = 0.44). Note that a Hazard Ratio of less than one indicates a better response for the experimental group. Sorafenib also improved overall survival (OS) from 15.2 months for the placebo and 17.8 months for the agent. The drug is also approved for the treatment of hepatocellular carcinomas and differentiated thyroid cancers.

Sunitinib is an oral indole-pyrrole receptor protein-tyrosine kinase inhibitor (Fig. 1B) with antitumor and anti-angiogenic activity that targets VEGFR1/2/3, PDGFRA/B, Kit, RET, and Flt3 receptors [8,22,23]. A phase II clinical trial was performed on 63 people with metastatic renal cell carcinomas who had failed cytokine therapy with IL-2 or IFN-α. IL-2 promotes the development of helper, cytotoxic, and regulatory T cells along with natural killer cells; it is produced physiologically by activated $CD4^+$ and $CD8^+$ T cells. IFN-α proteins, which are produced mainly by plasmacytoid dendritic cells, are mainly involved in innate immunity against viral infection, increased class I MHC (major histocompatibility complex) expression, and activation of NK (natural killer) cells. Patients with good or intermediate prognosis received treatment in 6-week cycles, with 4 weeks on therapy and 2 weeks off therapy. The partial response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) was 40%. A significant number of subjects (27%)

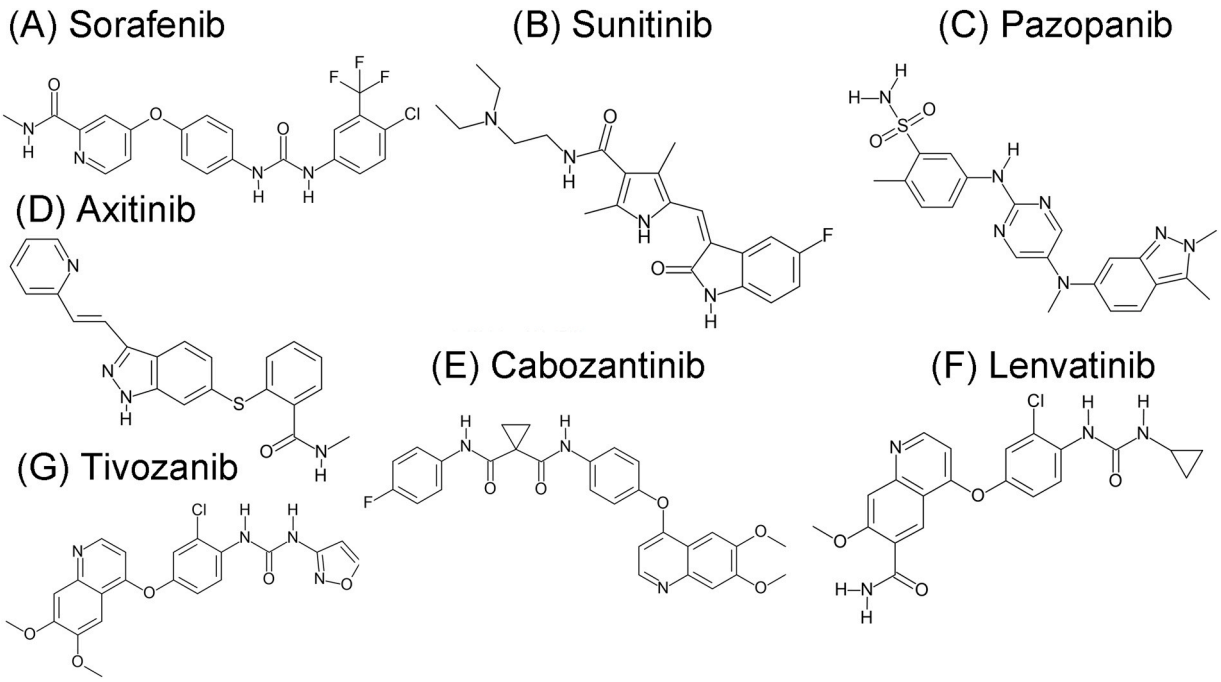

Fig. 1. Structures of selected FDA-approved small molecule protein kinase inhibitors used in the treatment of renal cell carcinomas.

experienced stable disease for more than 3 months while about one-third of them had either stable or progressive disease of less than 3 months duration.

In a second phase II trial involving 106 participants, a total of 44 (42%) patients achieved a partial response according to RECIST, including one complete response [23]. Overall tumor growth control (complete response, partial response, stable disease) occurred in 66% of the subjects and 24% of the participants had stable disease lasting more than 3 months. Fatigue (grade 2, 17%; grade 3, 11%), diarrhea (grade 2, 17%; grade 3, 3%), nausea (grade 2, 13%; grade 3, 0%), and stomatitis (grade 2, 9%; grade 3, 5%) were the most common side effects. Grades 3 and 4 neutropenia and hyperlipasemia complicated treatment in 15–20% of the subjects. These two consecutive phase II trials involving 169 participants demonstrated that sunitinib had substantial second-line antitumor activity for people with metastatic RCC. The response rate of 40–42%, a median progression-free survival (PFS) of 8.2 months, and a median overall survival (OS) of 16.4 months were particularly noteworthy when compared to prior studies in the second-line setting. Thus, inhibition of receptor-mediated signaling represents an appropriate therapeutic approach for refractory RCC. A phase III international randomized study of first-line therapy in 690 patients with metastatic disease comparing IFN-α with sunitinib further demonstrated the efficacy of sunitinib. On the basis of these findings, the United States Food and Drug Administration (FDA) granted approval for this drug for treatment of RCC in January 2006.

Pazopanib is an oral indazolylpyrimidine receptor protein-tyrosine kinase inhibitor (Fig. 1C) with antitumor and anti-angiogenic activity that targets VEGFR1/2/3, PDGFRA/B, and Kit [17,24–26]. A phase III study of pazopanib that led to its approval for the treatment of renal cell carcinomas was a placebo-controlled, randomized, double-blind, multicenter study. Participants were required to have clear cell or predominantly clear cell histology and could be either treatment naïve or have progressed on prior cytokine therapy. The primary endpoint of the study was progression-free survival. A total of 435 subjects were enrolled, with 290 and 145 patients in the pazopanib and placebo arms, respectively. The two arms were well matched with 53% and 54% of the participants being treatment naïve between the pazopanib and the placebo treated cohorts. Pazopanib significantly prolonged progression-free survival in comparison to the placebo (median PFS 9.2 months vs 4.2 months; HR = 0.46) among all participants. The difference was more pronounced between the subset of treatment-naïve participants (PFS 11.1 months vs 2.8 months) than those pretreated with cytokines (PFS 7.4 vs 4.2 months). The corresponding objective response rate (by independent review) was 30% vs 3% with a median duration of 14.6 months. Final overall data showed a median overall survival of 22.9 vs 20.5 months in the pazopanib and the placebo arms, respectively.

Axitinib is a potent indazole VEGFR1/2/3, PDGFRA/B, and Kit antagonist (Fig. 1D) that was approved for the treatment of advanced renal cell carcinomas in 2012 [11]. Rini et al. performed a randomized phase III study comparing axitinib, a selective second-generation inhibitor of VEGFR kinase activity, with sorafenib, an approved VEGFR protein-tyrosine kinase blocker, as a second-line therapy in individuals with metastatic renal cell cancers [27]. They included subjects aged 18 years or older with confirmed clear cell RCC who progressed despite first-line therapies containing sunitinib, bevacizumab plus INF-α, temsirolimus, or cytokines such as INF-α or IL-2. The primary endpoint was progression-free survival and this was assessed by a masked, independent radiology review panel. A total of 723 participants were enrolled and randomly assigned to receive axitinib (n = 361) or sorafenib (n = 362). The median PFS was 6.7 months with axitinib compared to 4.7 months with sorafenib (HR = 0.66) and the ORR was 19.4% vs 9.4% in these respective arms. Treatment was discontinued because of toxic effects in 14 (4%) of 359 people treated with axitinib and 29 (8%) of 355 subjects treated with sorafenib. The most common side effects were diarrhea, hypertension, and fatigue in the axitinib arm and diarrhea, palmar-plantar erythrodysesthesia (a skin reaction affecting the palms of the hand and soles of the feet and characterized by redness, swelling, and pain), and alopecia (hair loss) in the sorafenib arm of the trial. Axitinib resulted in significantly longer PFS compared with sorafenib. The authors concluded that axitinib is a bona fide treatment option for the second-line therapy of metastatic renal cell carcinomas.

Cabozantinib is an orally bioavailable, small-molecule quinoline VEGFR1/2/3, MET, Kit, FGFR1, RET, Axl, and Flt3 receptor protein-tyrosine kinase inhibitor (Fig. 1E) [17]. It was approved for the treatment of medullary thyroid cancers in 2012 and for renal cell carcinomas in 2016 [11]. An initial phase I study was undertaken using cabozantinib

in 25 heavily pretreated patients with metastatic renal cell cancers [28]. The most common side effects were fatigue (80%), diarrhea (64%), anorexia (36%), and vomiting (36%). Additionally, common grade 3 side effects included fatigue (20%), diarrhea (12%), pulmonary embolisms (12%), hypophosphatemia (40%), proteinuria (8%), decreased appetite (4%), and vomiting (4%). Of the 21 subjects assessable for radiological response by RECIST, a partial response was reported in 7 patients (28%) while 13 of them (52%) had stable disease and only one participant had disease progression. The median PFS was 12.9 months and the median OS was 15.0 months.

With robust phase I evidence of cabozantinib activity in renal cell carcinomas, investigators embarked on a phase III trial (METEOR) without conducting phase II trials [28]. The METEOR trial compared cabozantinib with everolimus, a macrolide (Fig. 2A) mTOR blocker, in patients with RCC who had experienced disease progression following treatment with a VEGFR protein-tyrosine kinase antagonist. Patient selection required (i) histological or cytological diagnosis of renal cell cancers with a clear cell component and (ii) recovery from prior toxicities related to any previous treatments. A total of 658 people were randomized in a 1:1 ratio to receive cabozantinib (330 patients) or everolimus (328 patients). The median patient age was 63 years (range, 31–86 years). All participants had received at least one prior VEGFR-targeted protein-tyrosine kinase blocker and had radiographic progression within 6 months after the most recent dose. Previous systemic therapy primarily consisted of sunitinib (62%), pazopanib (43%), or axitinib (16%). The primary endpoint of PFS was assessed on the first 375 subjects enrolled in the trial. In this portion of the study, 187 individuals were randomized to cabozantinib and 188 received everolimus. The median PFS almost doubled from 3.8 months with everolimus to 7.4 months with cabozantinib, representing a 42% reduction in the risk of progression or death. Cabozantinib was superior to everolimus for PFS across all subgroups. Objective tumor responses were observed in 21% of the cabozantinib group compared to 5% of the participants treated with everolimus. When further assessed by an independent radiology analysis, the median PFS across all enrolled participants was 7.4 months for the cabozantinib arm vs 3.8 months for the everolimus cohort, corresponding to a 48% reduction in the rate of disease progression or death with cabozantinib as compared with everolimus (HR = 0.58). The objective response rate was 17% for cabozantinib and 3% for everolimus. In the secondary endpoint of the trial, the entire study population of 658 individuals showed a median overall survival of 21.4 months with cabozantinib and 16.5 months with everolimus (HR = 0.66). The pivotal results of the METEOR trial led to the approval of cabozantinib by the FDA for the treatment of metastatic RCC in 2016.

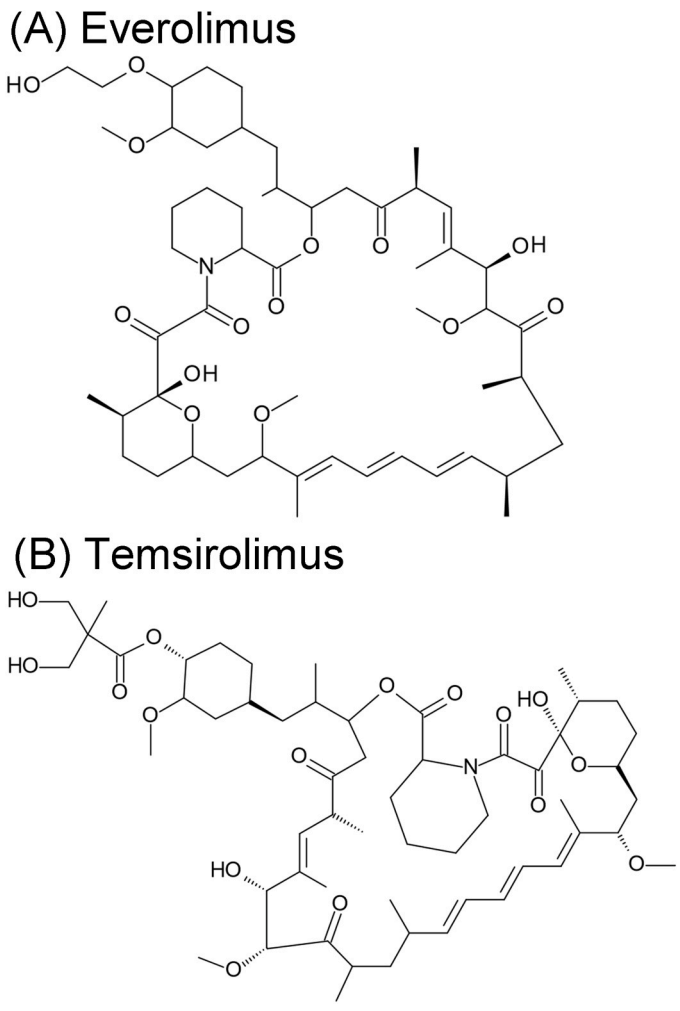

Fig. 2. Structure of macrolide drugs that inhibit mTOR protein-serine/threonine kinase and are FDA-approved for the treatment of renal cell carcinomas.

Motzer et al. assessed the efficacy of lenvatinib, everolimus, or their combination as second-line treatments in individuals with advanced renal cell carcinomas [29]. Lenvatinib is a quinoline-urea derivative (Fig. 1F) that blocks the activity of VEGFR1/2/3, FGFR1/2/3/4, PDGFRA, Kit, and RET. They performed a randomized, phase II, open-label, multicenter trial and enrolled patients with metastatic clear cell renal cell carcinomas. They included subjects who had received treatment with a VEGF-targeted therapy and progressed on or within 9 months of stopping that agent. Participants were randomized in a 1:1:1 ratio to either lenvatinib (24 mg/day), everolimus (10 mg/day), or lenvatinib plus everolimus (18 mg/day and 5 mg/day, respectively) administered orally in continuous 28-day cycles until disease progression or until unacceptable toxicity occurred. A total of 153 people was randomly allocated to receive either the combination of lenvatinib plus everolimus (n = 51), single-agent lenvatinib (n = 52), or single-agent everolimus (n = 50).

Lenvatinib plus everolimus significantly prolonged progression-free survival compared with everolimus alone (median 14.6 months vs 5.5 months; HR = 0.40) [29]. Moreover, the ORR was 43.1% with the lenvatinib/everolimus combination vs 6.0% with everolimus alone. Single-agent lenvatinib significantly prolonged PFS compared with everolimus alone (7.4 months vs 5.5 months; HR = 0.61). Grade 3 and 4 side effects occurred in fewer patients allocated single-agent everolimus (50%) compared with those assigned lenvatinib alone (79%) or lenvatinib plus everolimus (71%). The most common grade 3 or 4 treatment-emergent adverse event in individuals allocated lenvatinib plus everolimus was diarrhea (20%); in those assigned single-agent lenvatinib it was proteinuria (19%), and in those assigned single-agent everolimus it was anemia (12%). Lenvatinib plus everolimus and lenvatinib alone resulted in a progression-free survival benefit for people with metastatic renal cell carcinomas who had progressed after one previous VEGF-targeted therapy. Such studies lead to the FDA-approval in 2016 of the combination of lenvatinib plus everolimus as a second-line treatment of advanced RCC following one prior anti-angiogenic treatment.

Tivozanib is a quinoline-urea VEGFR1/2/3, Kit, and PDGFRB inhibitor (Fig. 1G) [30] that was approved for the treatment of refractory or relapsed renal cell carcinomas in 2021 following two or more systemic therapies [31,32]. Approval was based on the TIVO-3 study, a randomized trial of tivozanib vs sorafenib in subjects with metastatic RCC. Patients were randomized in this trial to receive either tivozanib (1.34 mg orally once daily for 21 consecutive days of every 28-day cycle) or sorafenib (400 mg orally twice daily without breaks). The primary endpoint was progression-free survival. The estimated median PFS was 5.6 months and 3.9 months in the tivozanib and sorafenib arms (HR = 0.73), respectively. The corresponding objective response rates

were 17.7% vs 8.0%. The most common grade 3–4 side effect in the tivozanib arm was hypertension (24%). Compared with sorafenib, tivozanib was associated with lower rates of grade 3–4 diarrhea, rash, and palmar-plantar erythrodysesthesia. Participants receiving tivozanib had lower rates of (i) dose reduction, (ii) interruption of therapy, or (iii) permanent discontinuation of the drug than those receiving sorafenib. In conclusion, tivozanib demonstrated efficacy when compared with sorafenib with an improvement in PFS.

mTOR is a key component of the intracellular signaling pathways involved in tumor cell growth, proliferation, survival, and angiogenesis [33]. Everolimus (Fig. 2A) and temsirolimus (Fig. 2B) are macrolide mTOR inhibitors that are approved for the treatment of renal cell carcinomas (www.brimr.org/PKI/PKIs.htm). The mTOR protein-serine/threonine kinase was identified first in yeast and subsequently in mammalian cells as the cellular target of rapamycin (sirolimus), which is a macrolide antibiotic that exhibits potent immunosuppressive activity [34]. mTOR has an evolutionarily conserved role in adjusting the nutritional status of the cell through its regulatory effects on messenger RNA (mRNA) translation. Treatment of cells with rapamycin inhibits mTOR kinase, resulting in the reduced translation of cell cycle regulatory proteins and an inability to progress from the G1 to the S-phase of the cell cycle. During malignant progression and tumorigenesis, mTOR activity may be increased by multiple signaling pathways including the phosphoinositide kinase/protein kinase B (PI3K/Akt) and Ras/mitogen-activated protein (MAP) kinase pathways, which are stimulated by overexpressed growth factors, their receptors, or mutations in upstream signaling molecules. Activated mTOR mediates the phosphorylation of translation-regulating factors including ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4EBP-1), thereby enhancing the translation of proteins that increase cell size, proliferation, and cell survival.

Hypoxia-Inducible Factor 1α (HIF-1α) is among the proteins affected by mTOR signaling; this transcription factor facilitates expression of genes needed for tumor cell growth in a hypoxic microenvironment [35]. Most renal cell carcinomas exhibit dysfunctional signaling pathways that either increase the activity of mTOR or depend upon mTOR activity for their pathogenesis. As noted previously, the Von Hippel-Lindau (*VHL*) gene, which targets HIF-1α for degradation by the proteosome, is silenced or mutated in up to 75% of sporadic clear cell RCCs. In such tumors, the increased level of HIF-1α plays a critical oncogenic role, including the increased transcription of VEGF, VEGFRs, or both. Activated mTOR exacerbates the loss of VHL function by further elevating HIF-1α through increased translation. Because dysregulated angiogenesis is a prominent feature of RCCs, the inhibition of mTOR is clinically important and may inhibit angiogenesis through mechanisms that differ from that of VEGFR-targeted agents. Temsirolimus, an intravenous mTOR inhibitor, was the first FDA-approved mTOR anticancer antagonist and it targets RCC (2007). Everolimus, an oral mTOR inhibitor, was initially developed in the organ transplantation setting, but appropriate dosing was established for patients with RCCs and its benefits were demonstrated in people with metastatic RCCs whose disease had progressed on VEGF-targeted therapy leading to its approval in 2009.

Rapamycin (sirolimus) and its congeners (everolimus, temsirolimus) inhibit mTOR by binding to a 12-kilodalton (kDa) cytosolic immunophilin receptor called FK506 binding protein 1 A, 12 kDa (FKBP-12) [34]. The resulting drug–FKBP-12 complex then inhibits the protein-serine/threonine kinase activity of mTOR complex 1 (mTORC1), which is composed of mTOR and several regulatory proteins. Rapamycin, everolimus, and temsirolimus are highly specific, potent inhibitors of mTORC1. A second mTOR complex (mTORC2) mediates the phosphorylation of Akt, but it is insensitive to inhibition by these agents.

Temsirolimus was the first mTOR inhibitor to demonstrate clinical benefit in subjects with metastatic renal cell carcinomas [36]. Intravenous temsirolimus results in high peak exposures thereby leading to significant tumor penetration. In a randomized phase III trial involving 626 participants, once-weekly intravenous temsirolimus prolonged the median overall survival of subjects with poor prognostic features by 49%, from 7.3 months in the IFN-α arm to 10.9 months in the single-agent temsirolimus arm (HR = 0.73). Single-agent temsirolimus also extended median PFS by 77%, from 3.2 months in the IFN-α arm to 5.6 months in the temsirolimus arm (HR = 0.74). The clinical benefit rate (objective response or stable disease for at least 24 weeks) was 32.1% in the temsirolimus group and 15.5% in the IFN-α group. Exploratory analyses indicated that the overall and progression-free survival benefits of temsirolimus were observed regardless of tumor histology (clear cell vs another histology – defined as indeterminate (12%) or non-clear cell (6%), including papillary and collecting duct carcinomas). Temsirolimus appears to be effective for both clear cell and non-clear cell histology and, thus, can be used for the treatment of all types of RCCs.

Motzer et al. reported the findings of a randomized phase III trial of everolimus (n = 277) vs placebo (n = 139) in individuals who had metastatic renal cell carcinomas with a clear cell component after progression on VEGFR protein-tyrosine kinase inhibitor therapy (sunitinib, 45%; sorafenib, 29%; both sunitinib and sorafenib, 26%) [37,38]. That study enrolled participants from all risk groups and other prior systemic treatments including IFN-α (51%), IL-2 (23%), chemotherapy (14%), and bevacizumab (9%). Treatment with everolimus in people with RCC after failure on VEGFR protein-tyrosine kinase inhibitor therapy resulted in a statistically significant improvement in progression-free survival compared with the placebo (HR = 0.3), as assessed by independent review. The median PFS was 4.9 months with everolimus vs 1.9 months for the placebo (HR = 0.33). Moreover, the progression-free survival benefit of everolimus was observed across all patient subsets, including those in all MSKCC (Memorial Sloan Kettering Cancer Center) prognostic risk groups and regardless of age, gender, or whether they had received previous treatment with sunitinib, sorafenib, or both. Additionally, about 10% of patients who received everolimus had side effects that led to discontinuation of therapy. Treatment-related grade 3 and 4 adverse events or laboratory abnormalities occurred in significantly more subjects on everolimus compared with those on the placebo and included lymphopenia (14% vs 5%); hyperglycemia (12% vs 1%); hypophosphatemia (4% vs 0%) and hypercholesterolemia, stomatitis, infections, and pneumonitis (all 3% vs 0%). Of the eight individuals who had grade 3 pneumonitis, a total of six discontinued everolimus treatment. Complete clinical resolution was observed in four of the eight patients while three of the eight subjects had improvement to grade 2 or less. Accordingly, the authors concluded that everolimus had an acceptable and manageable safety profile for people with metastatic RCC after progression on VEGFR protein-tyrosine kinase inhibitor therapy (sunitinib and /or sorafenib) [37,38].

2.3. *Structure of protein kinases and their interaction with inhibitory drugs*

As described originally for PKA (protein kinase A or cyclic AMP-dependent protein kinase) by Knighton et al., protein kinases such as that of the platelet-derived growth factor receptor protein-tyrosine kinase have a small N-terminal lobe and large C-terminal lobe (Fig. 3A) [39]. The small and large lobes form a cleft that serves as a docking site for ATP/ADP. A hinge region connects the two lobes. The amino-terminal lobe of protein kinases contains a conserved glycine-rich (GxGxΦG) ATP/ADP-phosphate–binding loop, which is the most flexible part of the lobe, where Φ is usually a hydrophobic residue. The 6-amino group of the adenine base of ATP/ADP forms a hydrogen bond with the first hinge residue (not shown). The β3-strand of all protein kinases typically contains an Ala-Xxx-Lys (AxK) sequence, the lysine of which forms a salt bridge with a conserved glutamate near the middle of the αC-helix (Fig. 3A). The presence of a salt bridge between the β3-lysine and the αC-glutamate is a prerequisite for the formation of active protein kinases. The amino-terminal lobe is made up of a five-stranded

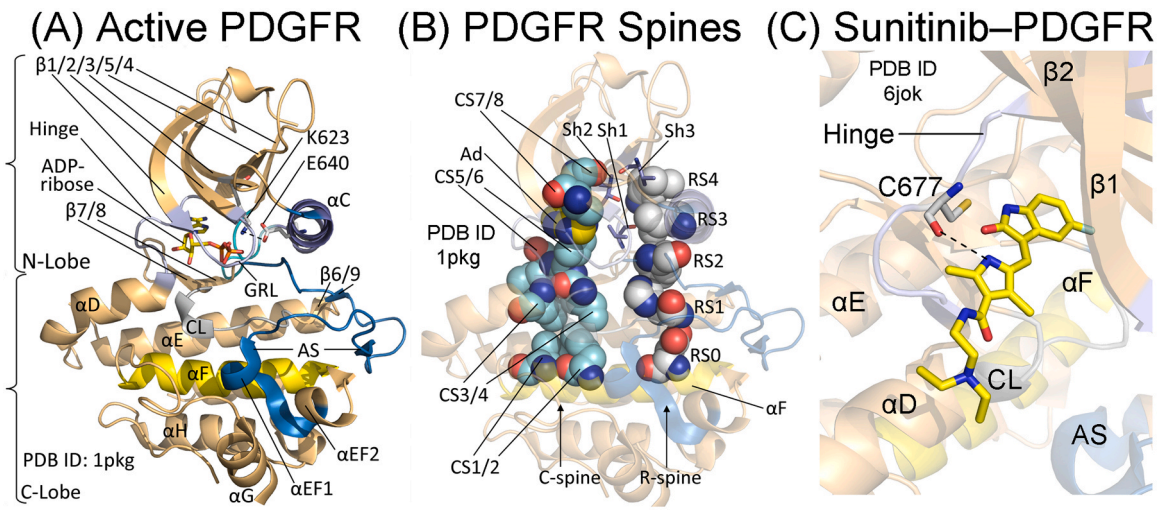

Fig. 3. (A) Overview of the structure of active platelet-derived growth factor receptor and (B) its C-spine and R-spine residues. AS, activation segment. GRL, glycine-rich loop. Figures 3–6 were prepared using the PyMOL Molecular Graphics System Version 1.5.0.4 Schrödinger, LLC.

antiparallel β-sheet (β1–β5) and an αC-helix [40,41]. The large lobe is mainly α-helical with eight conserved helices (αD–αI, αEF1, αEF2) [42]. The carboxyterminal lobe of catalytically active protein kinases also contains four short β-strands (β6–β9). Most small molecule protein kinase inhibitors make contact with many of the residues of the ATP-binding pocket within the cleft. For additional information on the structure of protein kinases, see Refs. [43,44].

Kornev et al. analyzed the three-dimensional structures of about two dozen active and inactive protein kinases to identify structurally and functionally important residues [45,46]. Their studies revealed a quartet of four amino acids that make up an R-spine (regulatory spine) and octet of eight amino acids along with the adenine base of ATP that make up a C-spine (catalytic spine). These spines produce a stable, but flexible, catalytically active ensemble. The C-spine positions ATP and the R-spine positions the protein substrate for catalysis. The R-spine contains components from both the αC-helix and the activation segment, whose configurations are important in determining active and dormant enzyme states. The R-spine contains the first residue of the β4-strand and the amino acid that is four residues C-terminal to the conserved αC-helix glutamate, both of which are within the small lobe [45]. The R-spine also contains the DFG-Phe of the activation segment, the HRD-His of the catalytic loop, and an aspartate residue within the αF-helix that interacts with the backbone N–H group of HRD-His, all within the large lobe. From the base to the apex, Meharena et al. labeled the R-spine residues as RS0, RS1, RS2, RS3, and RS4 [47]. We later labeled the C-spine residues from the bottom to the top as residues CS1–8 (Fig. 3B) [12]. We observed that the R- and C-spines of active protein kinases are linear. The exact positioning and alignment of both spines are necessary, but not sufficient, for the formation of catalytically competent protein kinases.

Based upon site-directed mutagenesis experiments, Meharena et al. found three residues in murine protein kinase A that strengthen and stabilize the regulatory spine, which they labeled as Sh1, Sh2, and Sh3 where Sh refers to shell [47]. A large amount of data indicate that many small molecule therapeutic steady-state ATP-competitive protein kinase inhibitors interact with the R-spine (RS2/3), the C-spine (CS6/7/8), and shell (Sh1/2) residues. The X-ray crystal structure of the sunitinib–PDGFR complex shows that the drug makes hydrophobic contact with CS6/7/8 and Sh1. The drug also interacts hydrophobically with L599 of the β-1 strand, K627 of the β-3 strand, ^{675}EYCFYG^{680} of the hinge-linker segment, and N644 within the αD-helix. Furthermore, the pyrrole N–H forms a hydrogen bond with the backbone carbonyl group of C677, the third hinge residue (Fig. 3C). For additional information on spine properties and their interaction with small molecule protein kinase inhibitors, see Refs. [10,17,32,48–50].

3. Treatment of renal cell carcinomas with monoclonal antibody targeted therapies

3.1. Structure of human IgG antibodies

Immunoglobulin G (IgG), which constitutes about 10–20% of plasma protein, is one of its most abundant components [51]. IgG is the major class of the five types of immunoglobulins in humans (IgM, IgD, IgG, IgA, IgE) and is the most abundant. The general characteristics of these five isotypes or classes are provided in Table 1. IgG consists of four subclasses: IgG1, IgG2, IgG3, and IgG4 in order of decreasing abundance. Although these four subclasses are more than 90% identical at the amino acid level, each subclass exhibits different properties in regard to antigen binding, complement activation, and interaction with receptors. Antibody responses to soluble protein antigens and membrane-associated proteins primarily induce IgG1. Responses to bacterial capsular polysaccharide antigens are generally restricted to IgG2. Viral infections lead to IgG antibodies of the IgG1 and IgG3 subclasses with the IgG3 antibodies appearing first during the course of infection. Allergens and helminth or filarial parasite infections are good inducers of IgG4. IgG1 and IgG3 are good activators of the complement response, IgG2 is a poor activator, and IgG4 fails to activate the complement response.

In their simplest form, e.g., IgG antibodies, each molecule is made up of two identical light chains (κ or λ) and two identical heavy chains that are linked by cysteine-derived disulfide (-S-S-) bonds [51]. Light chains consist of a variable region (V_L) on the amino-terminal half and a constant region (C_L) on the carboxyterminal half. Each of these segments is about 100 amino acid residues in length. Heavy chains consist of a variable amino-terminal portion (V_H) and three constant regions (C_H1, C_H2, C_H3). Each of these segments is also about 100 amino acids long. Each antigen binding site consists of residues derived from the variable region from one light and one heavy chain (Fig. 4). Each of these chains contains three complementary determining regions (CDRs) which correspond to the hypermutability portions of the immunoglobulin genes. CDR1 and CDR2 are found in the variable (V) region of a polypeptide chain and CDR3 includes some of V, all of diversity (D, heavy chains only) and joining (J) regions. CDR3 is the most variable. In a given mature B cell (plasma cell), only one heavy chain and one light chain allele are expressed so that antibodies with a single structure and specificity are produced.

Each of the immunoglobulin domains (V_L, C_L, V_H, C_H1, C_H2, C_H3)

Table 1
Properties of immunoglobulin subtypes.

Isotype	Heavy chains	Light chains	Antibody structure	Subtype	Secretory form	Function
IgA	α1, α2	κ or λ	$\kappa_2\alpha_2$ or $\lambda_2\alpha_2$	IgA1, IgA2	Monomer, dimer trimer	Important in saliva, tears, intestinal secretions, milk
IgD	δ	κ or λ	$\kappa_2\delta_2$ or $\lambda_2\delta_2$	None	None	Enhances mucosal homeostasis and immune surveillance
IgE	ε	κ or λ	$\kappa_2\varepsilon_2$ or $\lambda_2\varepsilon_2$	None	Monomer	Involved in allergic response and histamine release in asthma; atopic dermatitis
IgG	γ1, γ2, γ3, γ4	κ or λ	$\kappa_2\gamma_2$ or $\lambda_2\gamma_2$	IgG1/2/3/4	Monomer	Neutralizes toxins, viruses, and bacteria; can cross placenta; chief circulating antibody
IgM	μ	κ or λ	$\kappa_2\mu_2$ or $\lambda_2\mu_2$	None	Pentamer	First antibody expressed by each B cell

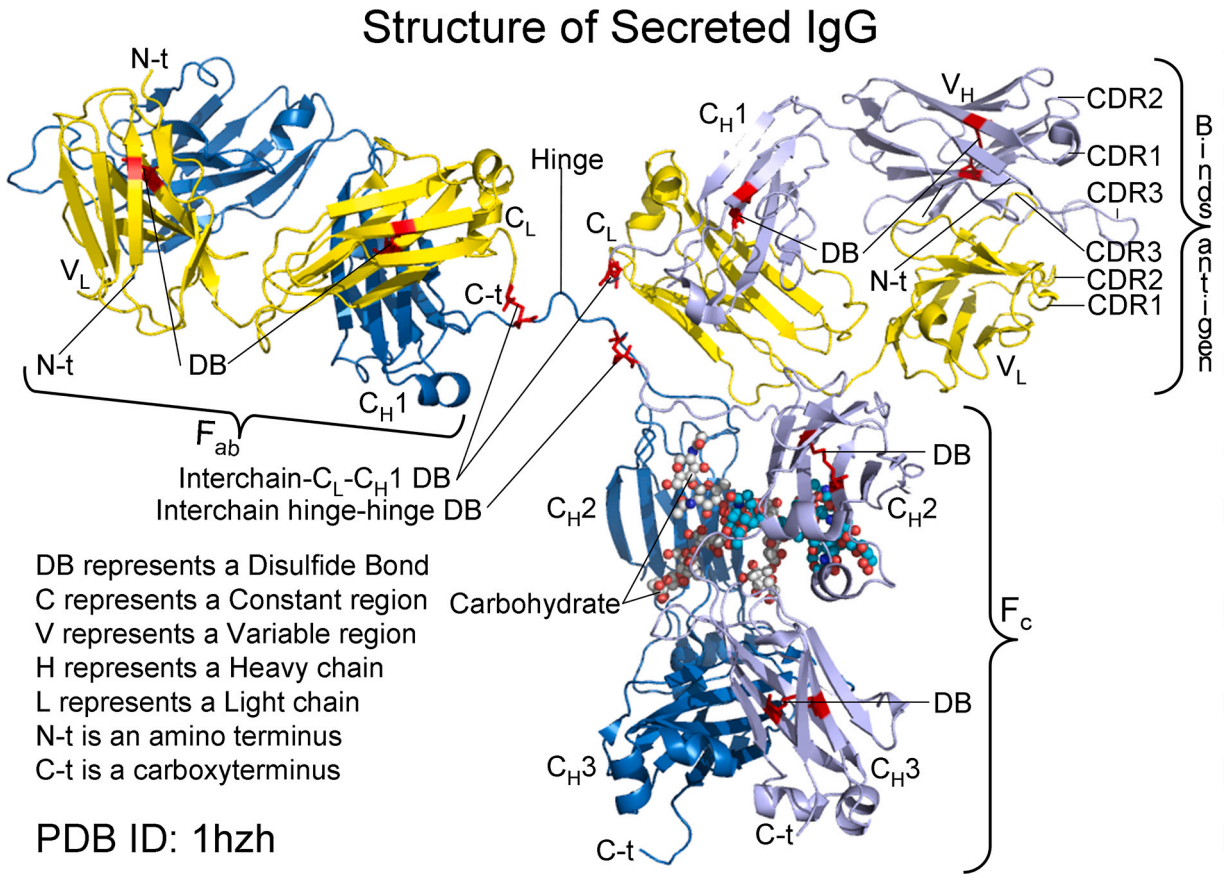

Fig. 4. A prototypical antibody structure. CDR, complementary determining region. This antibody targets human immunodeficiency virus.

forms a similar β-sandwich known as the Ig fold where the β represents β-strands [52]. The β-barrel consists of two sheets forming a sandwich-like structure. The variable domains consist of nine strands (ABCC'C''DEFG) and the constant domains are made of seven strands (ABCDEFG). In most antibody domains, a buried disulfide bridge derived from cysteines about 65 residues apart connects the B and F strands (Fig. 4). This bridge is approximately perpendicular to the individual sheets and helps to stabilize the folded domain. Proline residues are abundant in antibody domains and may contribute up to 10% of the amino acids. A prominent conserved *cis*-proline connects the B and C strands of the constant domains.

The two heavy chains are covalently attached by two interchain disulfide bonds [52]. Each of the variable and constant domains of the heavy and light chains contain intrachain disulfide bonds. The constant portions of the heavy chain are responsible for complement binding and for interacting with various membrane-bound Fc receptors (Fc refers to the fragment of the heavy chain portion of antibody molecules resulting from proteolytic hydrolysis of the hinge region in vitro; this fragment readily forms crystals, hence the c). Fab refers to the antigen-binding fragment of an immunoglobulin. The immunoglobulins are glycoproteins with carbohydrate chains covalently attached to an asparagine residue (N297) within the C_H2 domain. The core structure of the IgG glycans consists of *N*-acetylglucosamine and mannose residues. Other glycan components include galactose, sialic acid, and fucose. The C_H2 domains interact only by the *N*-linked glycans (Fig. 4). This region of the molecule also contains the binding site for the neonatal Fc receptor (FcRn), which is responsible for the (i) prolonged half-life of IgG, (ii) placental passage, and (iii) transport of IgG to and from mucosal surfaces.

The IgG hinge region forms a flexible linker between the Fab arms and the Fc fragment [51]. Owing to its flexibility, a portion of the hinge region in X-ray crystal structures may be absent owing to disorder (Fig. 4). The IgG1 hinge consists of about 15 amino acids. The IgG2 hinge contains 12 amino acids and is the shortest of all IgG subclasses. It contains a poly-proline helix that restricts the flexibility of these molecules. The IgG3 hinge at up to 62 residues is the longest of the four

subclasses, which is responsible for the higher molecular weight of this group. It contains 21 prolines and 11 cysteines forming a poly-proline helix with limited flexibility. The IgG4 hinge region consists of 12 amino acids. The relative flexibility of the Fab arms with respect to Fc ranks in the order IgG3>IgG1>IgG4>IgG2.

3.2. Treatment of renal cell carcinomas with monoclonal antibody immune checkpoint inhibitors

3.2.1. Pembrolizumab and axitinib or lenvatinib

Pembrolizumab is an IgG4 with a κ light chain targeting PD1 (program cell death protein 1) that is FDA-approved for the treatment of renal cell carcinomas, melanoma, and non-small cell lung cancer (NSCLC) [53]. It contains a hinge stabilizing S228P mutation. PD1 is an inhibitory immune transmembrane receptor of the CD28 family that modulates the activity of T cells in peripheral tissues [54]. It is expressed in T cells, many tumor infiltrating lymphocytes, natural killer cells, B cells, and monocytes. Under normal physiological conditions, the interaction of PD1 with its ligands (PDL1 or PDL2) prevents (i) excessive lymphocyte activation, (ii) excessive inflammation, (iii) destructive autoimmunity, and (iv) maintains immune tolerance to self-antigens by negatively regulating the immune response. PDL1 is often overexpressed by various tumor cells including those from lymphomas, melanomas, bladder and prostate cancers, NSCLC, and RCCs. Consequently, tumor cells attenuate T-cell signaling to evade immune surveillance. Blocking PD1–PDL1/2 interaction can restore T-cell activation leading to an anti-tumor response.

Na et al. determined the X-ray crystal structure of pembrolizumab bound to human PD1 [55]. PD1 consists of a canonical β-sandwich immunoglobulin variable topology with a disulfide bridge linking C54 and C123 (Fig. 5A). The pembrolizumab Fab in the complex possesses the β-sandwich immunoglobulin fold that closely resembles the full-length antibody (not shown). The interaction of PD1 with the antibody buries about 1770 Å^2 surface area. These authors describe two sub-interfaces between the antigen-antibody complex. Their sub-interface I involves the C'D loop of PD1 and the antibody Fab complementary determining regions LCDR1, LCDR3, and HCDR2. PD1 D85 forms a salt bridge with heavy chain R99 (not shown). Moreover, the R group of PD1 S87 hydrogen bonds with heavy chain R99. Additionally, PD1 E84, S87, Q88, and G90 hydrogen bond with light chain Y36 and heavy chain Y35, N59, and T58, respectively. The sub-surface II interactions include the R groups of PD1 N66 and K78 that hydrogen bond with the backbones of heavy chain R102 and Y101, respectively. The side chain of PD1 T76 hydrogen bonds with the R group of heavy chain Y101. Besides these polar links, Na et al. describe several hydrophobic interactions between residues of the antigen-antibody complex [55].

Pembrolizumab is FDA-approved for (i) the adjuvant treatment of people with renal cell carcinomas at intermediate-high or high risk of recurrence following nephrectomy or (ii) following nephrectomy and resection of metastatic lesions. It is also approved in combination with axitinib or in combination with lenvatinib for the first-line treatment of adults with advanced RCCs [4]. Axitinib is a selective second-generation VEGFR protein-tyrosine kinase antagonist whereas pembrolizumab is a monoclonal antibody that binds to PD1 (expressed on activated T cells) and blocks the interaction between PD1 and PDL1 or PDL2 ligands (expressed on tumor cells and antigen-presenting cells). Data from the randomized phase II KEYNOTE-426 trial resulted in the approval of

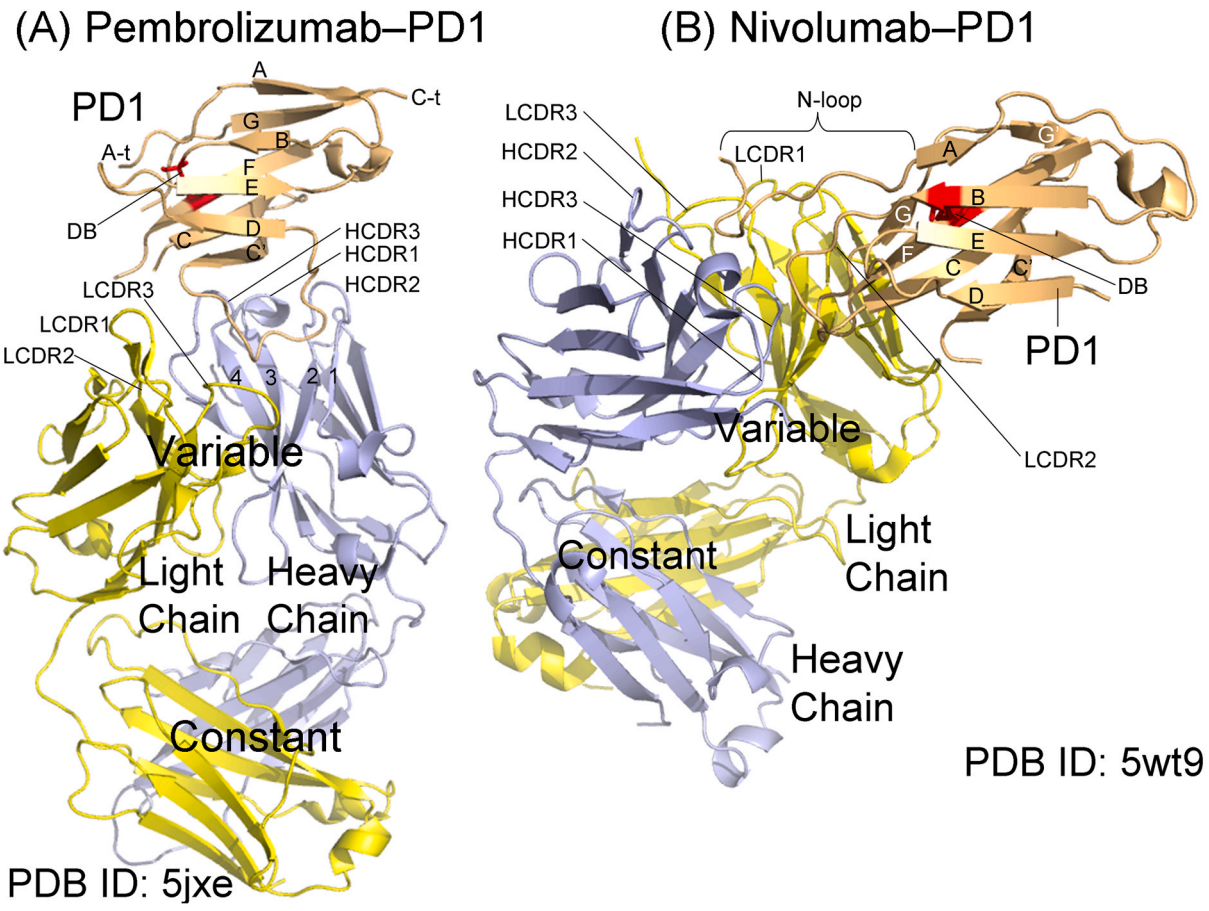

Fig. 5. (A) Structure of the pembrolizumab–PD1 complex. (B) Structure of the nivolumab-PD1 complex. A-t, amino-terminus; C-t, carboxyterminus; DB, disulfide bond; HCDR, heavy chain complementary determining region; LCDR, light chain complementary determining region.

axitinib in combination with pembrolizumab for the first-line treatment of participants with stage IV RCC. In the trial, which included 861 adults, the participants received the (i) therapeutic combination or (ii) sunitinib. Those receiving the combination regimen had a significantly higher objective response rate (59%) vs those receiving sunitinib (36%) and a longer median progression-free survival (PFS of 15.4 vs 11.1 months; HR = 0.71). A five-year follow up analysis continued to show a statistical benefit on OS and PFS for the pembrolizumab/axitinib group vs sunitinib [56]. The NCCN Guidelines include axitinib/pembrolizumab as a preferred first-line therapy option for individuals with clear cell RCC across all risk groups.

Lenvatinib is a multitargeted protein-tyrosine kinase inhibitor of VEGFR1/2/3, FGR1/2/3/4, PDGFRA/B, Kit, and RET (www.brimr.org/PKI/PKIs.htm). The randomized phase III CLEAR trial examined the efficacy of either lenvatinib/pembrolizumab, lenvatinib/everolimus, or sunitinib [4]. Those receiving lenvatinib/pembrolizumab had a longer median PFS of 23.9 months vs 9.2 months for sunitinib (HR = 0.39) and a higher ORR (71%) than those receiving sunitinib (36.1%). In a follow-up analysis, those receiving the lenvatinib/pembrolizumab combination had a median overall survival of 33.7 months vs 33.4 months for those receiving sunitinib (HR = 0.72). The NCCN panel recommends the lenvatinib/pembrolizumab as a preferred treatment option for people with clear cell RCC across all risk groups.

3.2.2. *Nivolumab with cabozantinib*

Nivolumab is an IgG4 with a κ light chain. It is an anti-PD1 immune checkpoint inhibitor that is FDA-approved for the treatment of renal cell carcinomas, melanomas, NSCLC, Hodgkin lymphomas, squamous cell carcinomas of the head and neck, colorectal cancers, hepatocellular carcinomas, and esophageal and gastric cancers [53,57]. Its mechanism of action is similar to that previously described for pembrolizumab. Tan et al. determined the X-ray crystal structure of nivolumab with PD1 [58]. PD1 is a type I transmembrane protein and its ectodomain contains a signal peptide, an N-loop, an IgV domain, and a stalk region. Like pembrolizumab, nivolumab uses both heavy and light chain residues to interact with PD1 with a buried surface area of 1932 $Å^2$. The interaction of PD1 involves HCDR1/2/3 and LCDR1/2 (Fig. 5B). The binding of the antibody to PD1 involves residues in the N-loop and residues of the FG and BC loops of the IgV PD1 domain. The N-loop contributes the majority of hydrogen bonds (10 of 16) within the antibody–PD1 complex. Specifically, L25, S27, P28, D29, and R30 of the N-loop form 10 hydrogen bonds with S30, N31, and G33 of HCDR1 and W52, Y53, and K57 of HCDR2 (not shown). The FG loop of PD1 forms five hydrogen bonds with D100 and D101 of HCDR3 and Y49 and T56 of LCDR2. T59 of the BC loop forms one hydrogen bond with N31 of HCDR1. In contrast to the pembrolizumab-PD1 complex, a major observation in this study was that the N-loop of PD1 was the prime component interacting with nivolumab.

Cabozantinib is multitargeted protein-tyrosine kinase antagonist of VEGFR1/2/3, FGFR1/2/3/4, Met, and Axl (www.brimr.org/PKI/PKIs.htm). It is FDA-approved in combination with nivolumab for the treatment of stage IV renal cell carcinomas based upon results from the randomized phase III CheckMate 9ER trial [4]. In this study involving 651 participants, the median PFS of the cabozantinib/nivolumab cohort was 16.6 months while that of the sunitinib group was 8.3 months. The ORR was 55.7% for the combination group and 27.1% for the sunitinib group. In a three-year updated analysis, median OS was 49.5 months for the cabozantinib/nivolumab group and 35.5 months for the sunitinib group (HR = 0.70). The median duration of response was 22.1 months for the combination group and 16.1 months for the sunitinib group. Cabozantinib/nivolumab is a preferred first-line therapy option for people with clear cell RCC across all risk groups according to the NCCN Guidelines.

3.2.3. *Ipilimumab with nivolumab*

Ipilimumab is an IgG1 antibody with a κ light chain targeting CTLA-4 (cytotoxic T-lymphocyte antigen 4) that is FDA-approved as (i) a single agent or (ii) in combination with nivolumab for the treatment of renal cell carcinomas, melanomas, colorectal cancers, hepatocellular carcinomas, NSCLC, malignant pleural mesotheliomas, and esophageal cancers [53,59]. CTLA-4 is a member of the CD28–B7–1/2 immunoglobulin superfamily of immune regulatory molecules; CTLA-4 functions as a negative regulator of T cell activation, particularly in CD28-dependent T cell responses [54]. The exhaustion or activation of T cells depends on the co-stimulatory and co-inhibitory signaling pathways that involve immune checkpoint molecules. Initially, B7–1 or B7–2 interact with naïve T lymphocytes via CD28 to activate them. This is followed by the inhibitory action of CTLA-4 that attenuates additional T cell activation by binding to the B7–1 (CD80) or B7–2 (CD86); B7–1/2 bind to CTLA-4 with substantially higher binding affinity than they bind to CD28.

Naïve T lymphocytes within lymph nodes need at least two signals to induce their proliferation and differentiation into effector and memory cells: Signal 1 is the antigen and signal 2 is provided by co-stimulators that are expressed on the antigen-presenting cells, typically as part of the innate immune response to microbes or to damaged host cells. CD28 is the chief activating receptor for B7–1/2 co-stimulators and CTLA-4 is a high-affinity receptor that binds and removes B7–1/2 from the surface of the antigen-presenting cells. Consequently, when CTLA-4 is expressed, B7–1/2 is reduced on the surface of the antigen-presenting cells and CD28 cannot be engaged and T cells do not receive adequate signal 2; accordingly, they cannot respond properly to antigens. Activation of CD8 cells by B7–1/2 or inhibition by CTLA-4 occurs within the lymph node. Activated CD8 cells leave the lymph node via the circulatory system and are carried to peripheral tissues. Activated CD8 cells express PD1 and their target cells express PDL1/2. This interaction reduces the killing of its target cells, including tumor cells. Antigen recognition without co-stimulation leads to unresponsiveness in T cells or anergy. Blockade of the CTLA-4 brake enhances immune responses and can lead to the destruction of tumors. James P. Allison received the Nobel Prize in Medicine or Physiology in 2018 for his work on CTLA-4 (www.nobelprize.org/uploads/2018/10/allison-lecture.pdf); he shared the prize with Tasuku Honjo who investigated the action of PD1 (www.nobelprize.org/uploads/2018/10/honjo-lecture.pdf).

He et al. determined the X-ray crystal structure of the ipilimumab–CTLA-4 complex (PDB ID: 5xje) [59] and Christ et al. also determined its structure (PDB ID: 6rp8, unpublished). We have used the latter coordinates for our analysis (Fig. 6A). The extracellular portion of CTLA-4 possesses the immunoglobulin fold that is made up of two β-sheets or a β-barrel: the front sheet closest to the antibody consists of A'GFCC' and the back sheet consists of ABED. As is the usual case, both the light and heavy chains participate in binding to the antigen (CTLA-4) and result in a buried surface of 1709 $Å^2$. HCDR2 and HCDR3 of the V_H domain and LCDR3 of V_L provide the major contacts to CTLA-4 with minor contributions from LCDR1 and LCDR2 but no contribution by HCDR1. Heavy chain Y53 (HCDR2) hydrogen bonds with the side chains of CTLA-4 residues R35, K95, and E97; heavy chain N57 (HCDR2) forms a hydrogen bond with the side chain of E33; heavy chain Y59 (HCDR2) forms a hydrogen bond with the carbonyl oxygen of M99; heavy chain W101 (HCDR3) hydrogen bonds with the S31 and K95 side chains. Light chain Y33 (LCDR1) forms a hydrogen bond with the CTLA-4 M3 carbonyl oxygen and the I108 N–H group; light chain G93 (LCDR3) forms a hydrogen bond with the N–H of L106; the backbone residues of light chain S95 (LCDR3) forms two hydrogen bonds with the backbone residues of Y104. There are also numerous hydrophobic interactions between the antibody and CTLA-4.

The phase III CheckMate 214 trial compared combination ipilimumab/nivolumab followed by (i) nivolumab monotherapy or (ii) sunitinib monotherapy in people with metastatic renal cell carcinomas [60,61]. Participants were randomized to receive nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) every 3 weeks for 4 cycles, then either nivolumab monotherapy or sunitinib (50 mg) daily (four 6-week cycles). In the intermediate and poor risk population, which were the cohorts in the

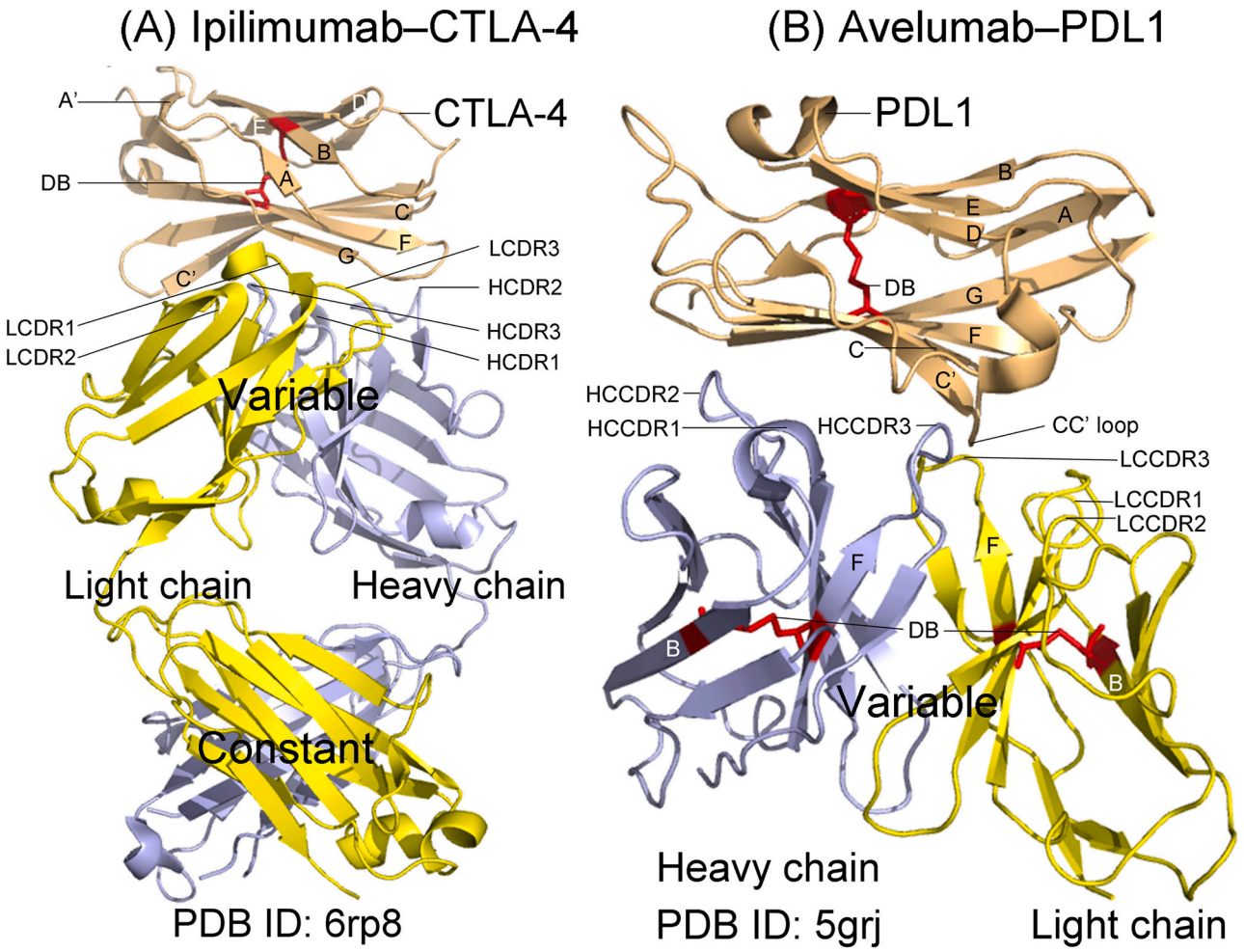

Fig. 6. (A) Structure of the ipilimumab–CTLA-4 complex. (B) Structure of the avelumab–PDL1 complex. DB, disulfide bond; HCDR, heavy chain complementary determining region; LCDR, light chain complementary determining region.

intended analysis, median OS was 47 months for ipilimumab/nivolumab vs 26.6 months for sunitinib (HR = 0.68). After a median follow-up of 67.7 months, median OS for all risk groups (favorable, intermediate, and poor) was 55.7 months for the combination group vs 38.4 months for the sunitinib group (HR = 0.72), median PFS was identical at 12.3 vs 12.3 months, and the ORR was 39.3% vs 32.4%. For patients with favorable risk, median OS was 74.1 months for the combination group vs 68.4 months for the sunitinib arm and the objective response rate and median progression free survival were lower in those receiving combination therapy (29% and 15.3 months, respectively) vs sunitinib (52% and 25.1 months, respectively). However, a higher proportion of patients achieved a complete response with ipilimumab/nivolumab compared with those who received sunitinib regardless of their risk categorization. The NCCN Guidelines continue to recommend the combination of ipilimumab/nivolumab as a first-line preferred regimen for the treatment of clear cell RCC of intermediate or poor risk individuals [4].

3.2.4. *Axitinib with avelumab*

Avelumab is an IgG1 monoclonal antibody with a λ light chain targeting PDL1 that is approved as a first-line therapy in combination with axitinib for the treatment of stage IV renal cell carcinomas [4,53]. The antibody contains a stabilizing S228P mutation. This antibody is also approved for the first-line treatment of metastatic Merkel cell carcinomas (neuroactive skin cancers) and for the second-line treatment of people with metastatic urothelial carcinomas (urothelial cells line the urethra, bladder, ureters, and renal pelvis). For RCC, 800 mg of avelumab is given intravenously every two weeks while 5 mg of axitinib is given orally twice daily. As noted previously, PDL1 is often overexpressed in a variety of tumor cells and blockade of PD1/PDL1 signaling can restore T cell activity and produce an anti-tumor response. Avelumab, an IgG1 antibody, actively participates in antibody-dependent cell-mediated cytotoxicity [62].

Liu et al. determined the X-ray crystal structure of avelumab with PDL1 [62]. PDL1 consists of two immunoglobulin domains: an N-terminal IgV domain and a C-terminal IgC domain (Fig. 6B). As described for the other monoclonal antibodies, both the heavy and light chains of avelumab are involved in binding to its PDL1 target and this interaction involves the IgV domain. Liu et al. report that this interaction results in a buried surface area of 1856 $Å^2$ [62]. They reported that this interaction involves five of the six complementary-determining regions of V_H and V_L with the three HCDRs predominating with minor contributions by LCDR1 and LCDR3. The avelumab-binding epitope on PDL1 occurs mainly via the C, C', F, and G strands, and the CC' loop. The CC' loop interacts with the CDR3 loops from both V_H and V_L of avelumab involving multiple hydrogen bond interactions. PDL1 residue D1 forms a hydrogen bond with R99 from LCDR3 loop and four hydrogen bonds with HCDR3 loop residues V104, T105, and T106. The buried surface of the C, C', F, and G strands is chiefly occupied by the HCDR2 and HCDR3 loops. HCDR2 residues Y52, S54, G55 and LCDR3 residue S97 form hydrogen bonds with PDL1 residues Y56, E58, N63, V76, R113, and S117.

Data from the randomized phase III JAVELIN Renal 101 trial involved participants with favorable, intermediate, and poor-risk subjects with renal cell carcinomas [63]. Motzer et al. randomly assigned

participants in a 1:1 ratio to receive avelumab (10 mg per kilogram of body weight) intravenously every 2 weeks plus axitinib (5 mg) orally twice daily or sunitinib (50 mg) orally once daily for 4 weeks (6-week cycle) [63]. The two independent primary end points were progression-free survival and overall survival among participants with programmed death ligand 1 (PDL1)–positive tumors. A total of 886 adults were assigned to receive avelumab plus axitinib (442 patients) or sunitinib (444 patients). Among the 560 subjects with PDL1–positive tumors (63.2%), the median PFS was 13.8 months with avelumab plus axitinib, as compared with 7.2 months with sunitinib (HR for disease progression or death, 0.61). In the overall population, the median PFS was 13.8 months, as compared with 8.4 months with sunitinib (HR = 0.69). Among the participants with PDL1–positive tumors, the objective response rate was 55.2% with avelumab plus axitinib and 25.5% with sunitinib. At a median follow-up for overall survival of 11.6 months and 10.7 months in the two groups, a total of 37 people in the combination group and 44 people in the sunitinib group had died. Progression-free survival was significantly longer with avelumab plus axitinib than with sunitinib among those who received these agents as first-line treatment for metastatic renal cell carcinomas. The NCCN Guidelines recommend the combination of axitinib/avelumab as a first-line regimen for the treatment of clear cell RCC across all risk groups [4]. For a list of the FDA-approved small molecule inhibitors and monoclonal antibodies used in the treatment of RCCs, see Table 2.

4. Epilogue

4.1. Evaluating the adverse events resulting from the systemic treatment of renal cell carcinomas

Prior to the development of small molecule receptor protein-tyrosine kinase inhibitors, the options and treatments of metastatic clear cell renal cell carcinomas were limited. As the previous paragraphs indicate, there are now many possible treatment modalities and the question arises on which treatment or treatments to choose and in what order. Krawczyk et al. studied the safety of protein kinase inhibitors that are currently approved as monotherapy or combination therapy for the treatment of advanced RCCs based upon randomized controlled clinical trials using a frequentist statistical approach [64]. Frequentist inference is a type of statistical algorithm based in frequentist probability, which treats probability in terms of frequency and draws conclusions from sample data by means of emphasizing a given frequency or proportion of findings in the data. The frequentist P score was used to determine the treatment ranking where a higher P score indicates a safer treatment (i.e., a lower risk of side effects).

Sorafenib and tivozanib used as monotherapy were the among best treatment options [64]. Sorafenib had the highest P score related to fatigue, nausea, vomiting, and hypertension, which means that this drug produced fewer of these side effects when compared with other therapies. Tivozanib had the highest P score for grade three or greater side effects, dose modification owing to side effects, and diarrhea. Sunitinib was the best treatment option in terms of diarrhea and dysphonia (hoarseness) of any grade. Cabozantinib, pazopanib, and the axitinib/pembrolizumab combination scored high in terms of the probability of minimizing nausea, vomiting, and fatigue. In contrast, the lenvatinib/pembrolizumab combination was the worst option in terms of all side effects, those side effects of grade three or more, and discontinuation or dose modification owing to side effects including nausea, vomiting, and fatigue. The axitinib/avelumab combination was the worst treatment option in terms of dysphonia, grade three or greater diarrhea, and hypertension. Furthermore, the cabozantinib/nivolumab combination was the worst option in terms of grade three or greater vomiting. Cabozantinib monotherapy had the lowest P score for diarrhea or hypertension of any grade. The anti-angiogenic actions of the small molecule protein-kinase inhibitors produce hypertension as one of the more common side effects. Although hypertension is usually an

Table 2
Drugs approved by the FDA for the treatment of renal cell carcinoma and other tumors[a].

Drug	Trade name	Company	Indications	Targets
Small molecule inhibitors				
Axitinib	Inlyta	Pfizer	RCC	VEGFR1/2/3, PDGFRA/B, Kit
Belzutifan	Welireg	Merck	RCC	HIF-2α inhibitor
Cabozantinib	Cabometyx	Exelixis, Inc.	RCC, HCC, medullary thyroid cancer	VEGFR1/2/3, MET, Kit, FGFR1, RET, Axl, Flt3
Everolimus	Afinitor	Novartis	RCC, HER2-negative breast cancer, PNET, renal angiomyolipoma, subependymal giant cell astrocytoma	FKBP12/mTOR
Lenvatinib	Lenvima	Eisai Limited	RCC, HCC, differentiated thyroid cancer, endometrial carcinoma	VEGFR1/2/3, FGFR1/2/3/4, PDGFRA, Kit, RET
Pazopanib	Votrient	Novartis	RCC, soft tissue sarcomas	VEGFR1/2/3, PDGFRA/B, Kit
Sorafenib	Nexavar	Bayer	RCC, HCC, differentiated thyroid cancer	VEGFR1/2/3, B- and C-Raf, Kit, Flt3, RET, PDGFRA/B
Sunitinib	Sutent	Pfizer	RCC, PNET, GIST	VEGFR1/2/3, PDGFRA/B, Kit, RET, Flt3
Temsirolimus	Torisel	Wyeth	RCC	FKBP12/mTOR
Tivozanib	Fotivda	AVEO	RCC	VEGFR1/2/3, Kit, PDGFRB
Monoclonal antibodies				
Avelumab	Bavencio	EMD Serono	Combination RCC therapy with axitinib, Merkel cell carcinoma, urothelial carcinoma	PDL1
Bevacizumab	Avastin	Genentech	Breast, cervical, colorectal cancers	VEGFA
Ipilimumab	Yervoy	BMS	RCC, CRC, HCC, NSCLC, mesothelioma, esophageal cancer	CTLA-4
Nivolumab	Opdivo	BMS	RCC, melanoma, NSCLC, mesothelioma, HCC, gastric cancer, head and neck squamous cell carcinoma, esophageal cancer, Hodgkin lymphoma, CRC, urothelial cancer	PD1
Pembrolizumab	Keytruda	Merck	RCC, melanoma, NSCLC, mesothelioma,	PD1

(*continued on next page*)

Table 2 (*continued*)

Drug	Trade name	Company	Indications	Targets
			HCC, gastric cancer, (i) head and neck and (ii) cutaneous squamous cell carcinoma, esophageal cancer, Hodgkin lymphoma, CRC, urothelial cancer, cervical, biliary tract, Merkel cell, endometrial, and triple negative breast cancers	

[a] CRC, colorectal cancer; GIST, gastrointestinal stromal tumors; HCC, hepatocellular carcinoma; HER2; human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; PNET, progressive neuroendocrine tumors of pancreatic origin; RCC, renal cell carcinoma.

unwanted result, it nevertheless serves as a potential marker of treatment effectiveness – it means the drug is working.

Since the KEYNOTE-426 study, the use of immune checkpoint inhibitors in combination with protein-tyrosine kinase inhibitors is now the standard of care for most adults with metastatic renal cell carcinomas [65]. The European Association of Urology guidelines for RCC recommends monotherapies only for those people who cannot receive or tolerate immune checkpoint inhibitors [66]. Although combination therapies may be more effective, there is usually an increase in unwanted side effects. A summary of front-line and second-line approaches for the treatment of renal cell carcinomas according to the NCCN Guidelines is given in Table 3. This list, however, includes monotherapies with cabozantinib and nivolumab. See Ref. [4] for the rationale and a comprehensive discussion of these recommendations.

4.2. Primary and acquired resistance of renal cell carcinomas to various treatment modalities

Despite the relative effectiveness of the above drugs, these treatments are not curative and the patient's disease progresses after varying lengths of time. The heterogeneous responses of people can be divided into three categories: (1) those with primary or intrinsic resistance who exhibit no or minimal initial response; (2) those who have secondary or acquired resistance and experience transient benefits followed by a relapse; (3) and a small subgroup for whom treatment proves effective over an extended timeframe [67]. In the case of renal cell carcinomas, the number of patients in the latter category is miniscule. In contrast, treatment of many patients with chronic myelogenous leukemia with BCR-Abl blockers results in a cure [68]. This diversity of patient outcomes underscores the multifaceted nature of resistance, which is due to factors such as genetic or epigenetic aberrations in cancer cells, environmental influences, and interactions with host cells [69–71]. These mechanisms can either manifest themselves before the start of therapy or they occur later. Each patient has a unique genetic profile, which requires a personalized therapeutic approach. However, the understanding of resistance requires a knowledge of the specific genetic landscape and the underlying mechanisms that determine adaptation to treatments with varying efficacy.

Table 3
Systemic therapy for metastatic clear cell renal cell carcinoma.[a]

First line therapy	
Recommended regimens	Useful in certain circumstances
Axitinib with pembrolizumab	Axitinib
Cabozantinib	Everolimus
Cabozantinib with nivolumab	Pazopanib
Ipilimumab with nivolumab	Sunitinib
Lenvatinib with everolimus	Tivozanib
Lenvatinib with pembrolizumab	Belzutifan[b]
Nivolumab	Bevacizumab
	High dose IL-2
	Temsirolimus
	Axitinib with avelumab
Second line therapy	
Axitinib	Axitinib with pembrolizumab
Belzutifan[b]	Cabozantinib with nivolumab
Cabozantinib	Everolimus
Lenvatinib with everolimus	Ipilimumab with nivolumab
Tivozanib	Lenvatinib with pembrolizumab
	Pazopanib
	Sunitinib
	Bevacizumab
	High dose IL-2
	Temsirolimus
	Axitinib with avelumab

[a] Adapted from Ref. [4].
[b] Belzutifan is a small molecule benzonitrile derivative that blocks HIF-2α binding to HIF-1β.

Soluble biomarkers have been evaluated as potential predictors of anti-angiogenic and immune checkpoint efficacy, including circulating cytokines and angiogenic factors [72]. Elevated peripheral cytokine concentrations of IL-6, IL-8, osteopontin, hepatocyte growth factor, and VEGF have been shown to forecast poorer clinical outcomes in patients receiving VEGFR protein-tyrosine kinase blockers. Elevated IL-6 and granulocyte/macrophage colony stimulating factor levels, both at baseline and on-treatment, predicted less responsiveness to these blockers. Similarly, on-treatment levels of peripheral serum inflammatory cytokines such as IFN-γ and IL-12 are correlated with clinical benefit from immune checkpoint inhibitors. However, these correlations need to be more rigorously substantiated and validated in future studies.

Because malignant transformation during the pathogenesis of renal cell carcinomas is a multipronged process, many genetic and cellular changes are involved and their order differs from tumor to tumor. It is unclear why some cancers respond to protein kinase or immune checkpoint monotherapies and others do not [60,70]. Resistance to receptor protein-tyrosine kinase inhibitors may be the result of tumor plasticity, activation of bypass pathways, tumor microenvironment interactions, and epigenetic modifications [70]. Resistance to the immune checkpoint inhibitors may result from defects in antigen presentation, a paucity of antigen-presenting dendritic cells, T-cell activity inhibition, the composition of the tumor microenvironment, gene mutations, and the gut microbiome composition. Other factors contributing to immunotherapy resistance include lack of IFN-γ pathway up-regulation and reduced response of the innate immune system [73]. For example, mutations in the interferon-receptor-associated *JAK1* (Janus kinase 1) or *JAK2* (Janus kinase 2) genes along with the concurrent deletion of the wild type allele can contribute to resistance. Also, a truncating mutation of the β2-microblobulin gene (*B2M*) can also lead to resistance. Although β2-microblobulin is not encoded by the *HLA* (human leukocyte antigen) gene, it is a component of the class I MHC antigen presenting apparatus.

There are several differences between CTLA-4 and PD1/PDL1 blockade [73]. Adverse events with anti-CTLA-4 therapy are frequent but with anti-PD1/PDL1 therapy they are less frequent. Overcoming toxicity resulting from the blockade of CTLA-4 is being addressed with different doses and dose regimens, particularly when combined with PD1/PDL1 blocking antibodies. Anti-CTLA-4 therapy targets the CD28 pathway, expands clonal diversity and primarily affects $CD4^+$ cells. Moreover, tumor recurrence is rare after partial or complete responses. In contrast, anti-PD1/PDL1 therapy targets the T cell receptor pathway, fails to expand clonal diversity, primarily affects $CD8^+$ cells, and activates exhausted $CD8^+$ cells. Additionally, tumor recurrence occurs more frequently after partial or complete responses to anti-PD1/PDL1 therapy. These immunotherapeutic agents can have additional anti-tumor activities when combined with anti-angiogenic treatments,

chemotherapy, or radiation.

Because tumorigenesis is an intricate process, it may be just as surprising that monotherapies are as effective as they are. The use of combination therapies with kinase and checkpoint inhibitors has been more effective than monotherapy alone, but this has been accompanied by an increase in toxicity. Despite these caveats, Hsieh et al. have referred to the present era (2015–2025) in the treatment of RCC as the golden age [3]. Moreover, they predict that we will enter the diamond era after 2025. We are hopeful that additional research and the further understanding of the pathogenesis of renal cell carcinomas will lead to improved therapies.

CRediT authorship contribution statement

Robert Roskoski: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The author is unaware of any affiliations, memberships, or financial holdings that might be perceived as affecting the objectivity of this review.

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