Primo N. Lara, Jr.
Eric Jonasch
Editors

Kidney Cancer

Principles and Practice

Second Edition

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ISBN 978-3-319-17902-5 ISBN 978-3-319-17903-2 (eBook)
DOI 10.1007/978-3-319-17903-2

Library of Congress Control Number: 2015942674

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

Important discoveries in the genetics and molecular biology of kidney cancer (or renal cell carcinoma) have driven the development of multiple new therapeutic approaches for patients with advanced disease. The characterization of the *VHL* gene as a regulator of angiogenesis has made renal cell carcinoma a paradigmatic disease for treatment with vascular endothelial growth factor pathway inhibitors. These agents are capable of inducing tumor shrinkage and of prolonging progression-free intervals in a significant number of patients. Inhibitors of the mTOR pathway have also demonstrated efficacy in individuals with this disease. In the United States (as of 2015), there are eight Food and Drug Administration-approved agents available for individuals with advanced renal cell carcinoma, including five antiangiogenic agents, two mTOR inhibitors, and one cytokine. However, it is clear that despite this relative wealth of new drugs, the vast majority of patients with advanced stage disease are not being cured. Much progress still needs to be made in our understanding of renal cell carcinoma biology and in the development of new therapy for both advanced disease and high-risk, nonmetastatic disease.

In this second edition of our textbook entitled *Kidney Cancer*: *Principles and Practice*, *w*e have once again brought together an internationally renowned multidisciplinary team of experts actively engaged in renal cell carcinoma research and/or clinical practice to write comprehensive chapters that cover every clinically relevant aspect of this disease. We continue with our key goal of making this textbook highly relevant to the practitioner, providing clinical vignettes, where appropriate, to illustrate how the chapter contents relate to the bedside. We include boxed sections that highlight the "Key Points" of that chapter. We hope that these boxed sections will serve as a quick reference to the busy clinician or enlightened lay person looking to find a bulleted summary of otherwise complex data. In addition, we provide both updated and new chapters on the genomics, genomic diversity, and molecular biology of renal cell carcinoma. We also emphasize some of the exciting new agents on the horizon that target and regulate immune checkpoints, as we anticipate these agents will play an important therapeutic role for patients with renal cell carcinoma in the near future.

It is our hope that this textbook will serve both as a reference work for the current state of the art in renal cell carcinoma treatment and a framework on which to build the next great advances in the management of this devastating disease. We are optimistic that the combined efforts of the broader research community will continue to move the field forward and that we will see major improvements in the treatment of renal cell carcinoma over the next decade.

This textbook would not have been possible without the administrative and editorial assistance provided by Springer, specifically Rosemarie Unger, as well as our diverse group of expert contributors. We also dedicate this textbook to our spouses (Elizabeth Lara and Anita Mahajan) and to our children (Joshua and Matthew Lara; Darius and Lucas Jonasch), who continue to provide both support and inspiration.

Eric Jonasch, MD
Primo N. Lara Jr., MD

Acknowledgments

The editors wish to acknowledge the following:

- Elizabeth, Matt, and Joshua Lara – for your never-ending love and support
- Anita, Darius, and Lucas – for your support and inspiration
- Gerald and Susan Knapp – for your generosity and commitment to translational research
- Merle Nery and the posse (Tonton and Mylene)
- To our patients – you are always in our hearts and minds

Contents

Part I

Epidemiology and Biology

Epidemiology of Renal Cell Carcinoma

1

Sujata Narayanan, Priti H. Patel, Alice Fan, and Sandy Srinivas

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Key Points

- Renal cell carcinoma (RCC) is the most common cancer arising from the renal parenchyma of clear cell histology.
- There is geographic variation in the rates of RCC with the highest incidence in North America and Europe and lower rates in Africa and Asia.
- In the United States, RCC rates have been increasing due mostly to diagnosis of early-stage tumors as a result of diagnostic imaging modalities.
- Cigarette smoking, obesity, and hypertension are well-established risk factors for RCC.
- Other risk factors including reproductive and hormonal factors, occupational exposures, and dietary habits have also been implicated, but the evidence remains inconclusive.
- Family history is associated with an elevated risk for RCC, and several genes have been identified through investigation of various inherited syndromes and have been targets for therapy.
- When clinicians encounter individuals with early-onset kidney cancer (age 46 years or younger), they should strongly consider referral for genetic counseling/germline mutation testing.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_1

1.1 Introduction

Renal cell carcinoma represents 4 % of all adult malignancies [1], and after prostate and bladder cancer, it is the third most common urologic tumor. The incidence of renal cell carcinoma varies substantially worldwide, with the developing countries having a higher incidence and mortality as compared to developing countries [2]. The cumulative risk of renal cell carcinoma is 1.06 % in developed countries and 0.3 % in developing countries [3]. In the United States (USA), it is estimated that 63,920 new cases of kidney and renal pelvis tumors were diagnosed in 2014 and approximately 13,860 would die of this disease [4]. It is the sixth most common cancer in men and the eighth most common cancer diagnosed in women in the United States [5].

Clear cell carcinoma of the kidney is the most predominant histological subtype derived from renal tubular cells. Other histopathological subtypes include the papillary, chromophobe, medullary, collecting duct, and spindle cell subtypes, among others. RCC presents in a sporadic or hereditary form, with the sporadic type occurring in the fifth decade or later in life and the hereditary form occurring in much younger patients.

Recently, a rising incidence of RCC has been noted and attributed to the increase in number of incidental tumors diagnosed as a result of more advanced imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) that are now available. These incidental tumors are more likely to be smaller in size and localized to the kidney. According to the SEER 9 Incidence and Mortality Data from 1975 to 2011, the overall survival from RCC has also increased, with the 5-year relative survival rates increased from 52.1 % in 1975 to 73.5 % in 2006 [6]. These improved survival rates also indicate the advances in the management of RCC in the past two decades with introduction of improved surgical techniques and systemic therapies for these patients.

1.2 Incidence and Demographics

1.2.1 Age

RCC is most frequently diagnosed among people aged 55–64, with a median age of diagnosis being 64 years [6]. According to the 2007–2011 SEER database, approximately 1.2 % of patients were diagnosed under age 20, 1.8 % between 20 and 34, 6 % between 35 and 44, 16.4 % between 45 and 54, 26.2 % between 55 and 64, 25.2 % between 65 and 74, 17.4 % between 75 and 84, and 5.7 % 85+ years of age [6].

1.2.2 Sex

The incidence of RCC is more common in men [6, 7] with a reported male to female ratio of 1.5:1 [6]. Recent analysis from the SEER database reported that men were diagnosed with larger tumors with a higher grade as compared to women [8]. Analysis of the SEER database also showed that the median overall survival from time of diagnosis was 130 months for females versus 110 months for males ($p<0.0001$). In comparison of males and females, 5-year cancer-specific survival was 78 % versus 81 %, and 5-year overall survival was 65 % versus 69 % ($p<0.0001$) [8].

1.2.3 Race

African-Americans have the highest incidence of RCC compared to other racial groups [6, 9, 10], along with a higher mortality rate secondary to this disease. In a retrospective cohort study done on patients in the SEER and Medicare databases, overall survival was worse for blacks than whites even after adjustment for demographic and cancer prognostic factors (hazard ratio [HR] = 1.16; 95 % CI, 1.07–1.25). This lower survival rate could be explained by the increased number of comorbid health conditions and the lower rate of surgical treatment among black patients [11].

Presentation with non-clear subtypes of RCC, such as papillary RCC, has been noted to be higher in African-Americans [12].

1.3 Risk Factors for Renal Cell Carcinoma

Several risk factors have been well established for renal cell carcinoma, including tobacco use, obesity, and hypertension, although the complexity of these associations and their mechanisms have yet to be elucidated (Table 1.1). Other risk factors such as reproductive and hormonal factors, occupational exposures, and dietary habits have also been implicated, but the evidence remains inconclusive.

Table 1.1 Risk factors for renal cell carcinoma

Established risk factors	Risk factors that need further study
Cigarette smoking	Dietary factors:
	Fruit and vegetables
	Alcohol
Obesity	Reproductive factors and other hormones:
	Oral contraceptive pills
	Parity
Hypertension	Occupational exposures:
	Asbestos, cadmium, hydrocarbons, gasoline, trichloroethylene
Inherited susceptibility	Analgesics

1.3.1 Cigarette Smoking

Cigarette smoking is considered a causal risk factor for renal cell carcinoma by both the International Agency for Research on Cancer and the US Surgeon General. Most case–control [13–15] and cohort studies [16–19] have reported significant associations between cigarette smoking and increased rates of renal cell carcinoma, with relative risks greater than 30 %. Studies have also shown significant dose–response trends with the number of cigarettes smoked [14, 20]. This observation, together with the decline in risk following cessation, supports causation between cigarette smoking and RCC [14, 15, 21]. A meta-analysis of 24 studies showed that compared with lifetime nonsmokers, smoking increased renal cell carcinoma risk by 54 % among men and 22 % among women [22]. A clear dose–response pattern of risk was apparent for men and women, with risk doubling among men and increasing 1.6-fold among women who were heavy smokers (>21 cigarettes per day). There was a significant 15–30 % reduction in RCC risk 10–15 years after smoking cessation, which was observed in both sexes.

The mechanism of carcinogenesis through cigarette smoke may be mediated by one of the constituents, *N*-nitrosodimethylamine, a nitroso compound. Renal cell carcinoma patients were shown to have a higher level of DNA damage in their peripheral blood lymphocytes induced by a tobacco-specific *N*-nitrosamine compared to control subjects [23]. In addition, this compound has caused renal tumors in several animal species. A further study revealed *N*-nitrosodimethylamine induced clear cell renal tumors in rats with VHL mutations suggesting a possible molecular pathway from tobacco smoking to RCC [24, 25]. Genetic alterations frequently found in RCC such as deletions in chromosome 3p were also shown to be more common in cultured peripheral blood lymphocyte cells from renal cell carcinoma patients than control subjects after being treated with benzo[α]pyrene diol epoxide, a major constituent of cigarette smoke [26].

NAT2, a gene encoding the *N*-acetyltransferase 2 enzyme that is involved in the metabolism of arylamine in tobacco smoke, has been evaluated in a few studies of renal cell carcinoma. Smoking-related RCC risk was higher in individuals with slow acetylator genotype for NAT2 than rapid acetylators [27]. This suggests that NAT2 is an underlying susceptibility marker for RCC that can exacerbate RCC risk in combination with risk factors such as cigarette smoking. In addition to carcinogens in tobacco smoke, cigarette smoking is hypothesized to increase renal cell carcinoma

risk through chronic tissue hypoxia caused by smoking-related conditions such as chronic obstructive pulmonary disease and exposure to carbon monoxide [28]. There is also evidence to suggest that passive exposure to cigarette smoke among nonsmokers as well as occasional smoking may increase the risk of renal cell carcinoma.

1.3.2 Obesity

The increasing prevalence of obesity is likely to account in part for the rising incidence of renal cell carcinoma. It has been estimated that over 40 % of renal cell carcinomas in the United States and over 30 % in Europe may be attributable to being obese and overweight [29–33]. The cumulative evidence from analytical epidemiologic studies is most consistent for obesity to be a risk factor for RCC in both women and men. A quantitative review of published studies showed that increased BMI was strongly associated with increased risk of RCC among men and women, after controlling for confounding factors [29]. A dose-dependent relationship exists as described in a meta-analysis of data from prospective observational studies which estimated that the risk of developing renal cell carcinoma increased 24 % and 34 % for men and women, respectively, for every 5 kg/m^2 increase in body mass index (BMI) [34].

Several plausible mechanisms by which obesity influences renal cell carcinoma development have been hypothesized, but the actual pathophysiology has not been fully elucidated. Obesity may promote changes in the hormonal milieu by altering circulating levels of estrogen and other steroid hormones, or elevated levels of insulin-like growth factor-I (IGF-I), which could in turn contribute to the development of renal cell carcinoma by affecting renal cell proliferation and growth [31, 35–37]. In obese individuals lipid peroxidation is increased leading to oxidative stress through the formation of DNA adducts which may promote the development of RCC [38].

Other proposed mechanisms include chronic tissue hypoxia, elevated cholesterol level and downregulation of low-density lipoprotein receptor, lower levels of vitamin D, and increases in adipose tissue-derived hormones and cytokines, such as leptin and adiponectin [33, 39, 40].

1.3.3 Hypertension

Hypertension can be the result of renin-producing tumors as well as from treatment of RCC with tyrosine-kinase inhibitors [41, 42]. Sufficient evidence from cohort studies has accumulated linking hypertension reported at baseline to subsequent renal cell carcinoma incidence [43–45].

Dose–response relations between measured blood pressure level and renal cell carcinoma risk have been reported [16, 46–49]. Compared to individuals with normal blood pressure, those with the highest blood pressure (100 mmHg diastolic pressure or 160 mmHg systolic pressure) were found to have twofold or higher risk. In a cohort of Swedish men with sequential blood pressure measurements during follow-up, the risk of RCC further increased among those whose blood pressure increased above the baseline level and reduced among those whose blood pressure declined over time [16]. This data suggests that hypertension could be a factor in renal cell carcinoma development, and the risk can be modified with better control of blood pressure.

In the United States, national surveys indicate that the prevalence of hypertension in the population has been increasing along with the number and types of medications used to treat hypertension. Most epidemiologic studies of antihypertensive drugs and renal cell carcinoma risk have found that diuretic use, a causal factor candidate in early studies, is not an independent risk factor and adjustment for high blood pressure appears to eliminate any excess risk associated with diuretic use [43, 49–51]. In a population-based evaluation of various antihypertensive medications in Denmark, excess risk of renal cell carcinoma was observed only during short-term follow-up, and risks were reduced to insignificant levels five or more years after the baseline [50]. Also in this study, no particular type or class of antihypertensive medication was consistently associated with renal cell carcinoma risk.

The association between hypertension and renal cell carcinoma risk has been shown to be independent of the effects of excess body weight and cigarette smoking [16, 43, 45, 47, 48, 52]. Individuals who are both obese and hypertensive have greater risk of developing renal cell carcinoma than those who have only one of these conditions [16, 48, 53].

The biologic mechanism underlying the association between hypertension and renal cell carcinoma risk has yet to be elucidated. Among the hypotheses proposed are lipid peroxidation and the formation of reactive oxygen species, which are elevated in hypertensive individuals and are thought to play a role in renal cell carcinoma development [38]. Chronic renal hypoxia accompanies hypertension and leads to the upregulation of hypoxia-inducible factors. In animal models this has been shown to increase proximal tubular cell proliferation and glomerular hypertrophy and may be a mediator in kidney oncogenesis [54–56].

1.3.4 Genetics

Renal cell carcinoma occurs in both sporadic and hereditary forms. However, sporadic renal cell carcinomas have been shown to have a familial predisposition, with a recent meta-analysis indicating a greater than twofold risk among individuals having a first-degree relative diagnosed with kidney cancer [57]. A study evaluating familial aggregation among RCC patients in Iceland demonstrated a two- to threefold increase in RCC risk for first-degree relatives and a 1.6-fold increased risk for third-degree relatives [58]. The interplay of exposures to environmental risk factors and genetic susceptibility of exposed individuals is believed to influence the risk of developing sporadic renal cell carcinoma.

Hereditary renal cell carcinoma tends to occur earlier in life than sporadic forms of the disease and often involves bilateral, multifocal tumors [59]. Only about 3–4 % of renal cell carcinomas are explained by inherited predisposition of familial cancer syndromes, most notably the von Hippel–Lindau (VHL) syndrome. This syndrome is characterized by alterations in the *VHL* tumor suppressor gene, located on chromosome 3p, which predisposes to the clear cell subtype of renal cell carcinoma. The carcinogenesis pathway involves the VHL protein forming an ubiquitin ligase complex with proteins including elongin C, elongin B, and Cul-2. This complex targets the hypoxia-inducible factor (HIF)-1α pathway for degradation [60–62]. The HIF regulates multiple downstream genes via the mitogen-activated protein kinase (MAPK) and mTOR pathways whose expression is increased when the VHL gene is inactivated. These genes include the vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF), which are critical in the pathway for tumorigenesis and are targets for therapeutic approaches for the treatment of renal cell carcinoma [63, 64]. Clinically, VHL is an autosomal dominant disorder characterized by clear cell RCC, retinal hemangiomata, cerebellar and spinal hemangioblastomas, pheochromocytomas, and pancreatic neuro-endocrine tumors [65].

There are other rare forms of renal cell carcinoma that have an inherited susceptibility (Table 1.2). Only a very small proportion of renal cell carcinoma patients are known to occur in families with these rare syndromes. Hereditary papillary carcinoma is an autosomal dominant syndrome where patients are at risk of developing bilateral multifocal type 1 papillary renal carcinoma, often at a late age of onset at 50–70 years [66]. Activation of a proto-oncogene, *MET* at 7pq31, is the inciting event, which activates downstream signaling cascades inducing cell proliferation and differentiation [67]. Birt–Hogg–Dubé syndrome is caused by abnormalities in the folliculin (FLCN) gene, an autosomal dominant tumor suppressor gene [68, 69]. Affected persons are at risk to develop cutaneous fibrofolliculomas, pulmonary cysts, spontaneous pneumothoraces, and renal tumors [70]. Renal lesions are bilateral and multifocal. The histological subtypes are usually chromophobe, oncocytic, or mixed [71]. Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a rare condition characterized by cutaneous and uterine leiomyomas [72]. Type II papillary renal cell carcinoma has been associated with HLRCC,

Table 1.2 Inherited syndromes associated with renal cell carcinoma

Syndrome	Genetic inheritance	Prevalence	Histology	Incidence	Mean age at diagnosis	Clinical features
Von Hippel–Lindau (VHL) [68]	VHL (3p25-26) Autosomal dominant	1 in 36,000	Cysts Clear cell	25–45 %	40	Retinal and CNS hemangioblastomas Pheochromocytomas Pancreatic cysts Pancreatic neuroendocrine tumors
Hereditary papillary renal cancer (HPRC) [69, 70]	MET (7q31) Autosomal dominant	Rare	Papillary type I	Unknown	>50	None
Birt–Hogg–Dubé (BHD) [71–74]	FLCN (17p11.2) Autosomal dominant	>60 families from various populations	Chromophobe Oncocytic Clear cell Papillary Mixed (chromophobe/oncocytic)	38 %	48	Fibrofolliculoma Trichodiscoma Acrochordon Lung cysts Pneumothorax
Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) [75–77]	FH (1q42–43) Autosomal dominant	>100 families from various populations	Papillary type 2 Collecting duct	2–16 %	44	Cutaneous and uterine leiomyomas
Tuberous sclerosis [79–81]	TSC1 (9q34) TSC2 (16p13) Both AD	1 in 6,000	Angiomyolipoma Renal cysts Clear cell Chromophobe Oncocytoma Papillary	2–3 %	36	Epilepsy Mental retardation Adenoma sebaceum Hypomelanotic maculae Shagreen patch Fibrous plaques Ungual fibroma Dental pits Cardiac rhabdomyoma Periventricular hamartomas (tubers)
Hereditary paragangliomas (PGL) [82–84]	SDHB (1p36) SDHC (1q21) SDHD (11q23) All AD	Unknown	Clear cell	3 cases in 2 families	30	Paragangliomas Pheochromocytomas

with an onset of 30–50 years of age. These renal cancers are usually unilateral and often aggressive leading to death from metastatic disease within 5 years of diagnosis [73]. A mutation in the fumarate hydratase gene located on chromosome 1, an autosomal dominant tumor suppressor gene, leads to transcriptional upregulation of HIF target genes [74]. Some families with clear cell cancer have a balanced translocation involving chromosome 3 [75]. Tuberous sclerosis is an autosomal dominant disorder characterized by hamartomas in various organs. Other features can include epilepsy and cutaneous manifestations such as hypomelanotic macules, facial angiofibromas, shagreen patches, and ungual fibromas [76]. Tumor suppressor genes TSC1 and TSSC2 encoding hamartin and tuberin, respectively, are involved in regulation of the mTOR pathway and have been linked to tuberous sclerosis [77]. Renal manifestations include multifocal clear cell renal cancers and angiomyolipomas which can be large requiring surgical removal [78]. Hereditary paraganglioma (HPG) is an autosomal condition caused by a mutation in genes encoding mitochondrial succinate dehydrogenase (SDHB) [79]. There are reports of an increased incidence of clear cell renal cancer in two families with HPG because of a SDHB mutation although other histologies have also been described [80, 81]. Genetics plays an integral role in the inherited susceptibility of renal cell carcinoma; however, it has been shown that the majority of non-inherited clear cell carcinomas are associated with inactivation of the VHL gene through mutation or promoter hypermethylation [59]. Another example of a non-inherited genetic mutation is Xp11 translocation renal cell carcinoma (RCC), an RCC subtype that was introduced in 2004 as a genetically distinct entity into the World Health Organization classification of renal tumors. It accounts for at least one-third of pediatric RCCs and for 15 % of RCCs in patients <45 years of age. It is characterized by Xp11.2 translocations, which induces gene fusions involving the TFE3 transcription factor gene. Xp11 translocation RCC usually has a mixed papillary architecture with nested patterns of clear and/or eosinophilic cells and calcified foci. Patients with metastatic Xp11 translocation RCC have presented at an advanced stage and are usually afflicted by a short and aggressive disease course [82, 83].

Detection of hereditary forms of RCC relies on the clinician to appropriately recognize individuals with potentially inherited forms of cancer. Since the diagnosis of hereditary syndromes can have far-reaching consequences for the patients and their families, it has now been suggested that clinicians refer patients with early-onset kidney cancer (age 46 years or younger) for genetic counseling and germline mutation testing [84].

1.3.5 Hormonal and Reproductive Factors

Reproductive and hormonal factors may play a role in renal cell carcinoma development in susceptible individuals. Tissue from renal cell carcinoma patients has been shown to express steroid hormone receptors and luteinizing hormone-releasing hormone receptors [85, 86]. In animal studies, estrogen treatment has been shown to enhance the development of renal cell carcinoma, whereas removal of the ovaries reduced neoplastic renal changes [87]. An increased risk of renal cell carcinoma has been associated with parity among women in several studies. Compared with nulliparous women, the risk of renal cell carcinoma increased 40–90 % among women who had given birth [88–90]. A Swedish study found a significant 15 % increase in risk with each additional birth, after controlling for age at first birth among parous women [90]. An inverse association with age at first birth has also been reported, with highest risk among women who gave multiple births at a relatively young age [91]. Mechanisms underlying the observed association with parity are unclear, although pregnancy-induced hypertension and renal stress may play a role. Associations with other reproductive-related factors, including the use of oral contraceptives, which in some studies has been shown to be protective, and hormone replacement therapy, are not consistently observed [53, 92, 93].

1.3.6 Occupational and Environmental Exposures

Generally, renal cell carcinoma is not considered an occupational disease, but it has been linked to some occupations and industrial exposures. Trichloroethylene (TCE), a chlorinated solvent used as a degreaser in metal industries and as a general solvent, has been the most extensively studied risk factor for renal cell carcinoma. Three studies were initiated in response to a cluster of renal cell carcinoma cases observed in a plant in Germany. All of these studies reported elevated relative risks for renal cell carcinoma associated with TCE exposure [94]. Although not statistically significant, aerospace workers with airborne TCE exposures above 50 ppm were at a near twofold risk of kidney cancer mortality compared with workers exposed to lower levels [95]. In contrast, no association was reported in a small cohort study of TCE-exposed workers in Denmark and another retrospective cohort mortality study of workers exposed to chlorinated organic solvents in Taiwan [96, 97]. Given the methodological challenges including the complexities of TCE pharmacokinetics, co-exposure to other solvents, various study limitations, and the lack of association in some reports, further studies are warranted before causality is implicated [98–102]. Environmental carcinogen exposures may be linked to tumor DNA alterations. RCC patients with high, cumulative exposures of trichloroethylene have been shown to have more frequent somatic VHL mutations. A German study reported that *VHL* mutations were found in 33 of 44 RCC patients with TCE exposure. Of the 33 patients with *VHL* mutations, 14 had multiple VHL mutations and 13 had the same C to T substitution in codon 81 [103]. Genes encoding the glutathione S-transferase (GST) enzymes, including *GSTM1*, *GSTT1*, and *GSTP1*, have been studied in relation to renal cell carcinoma risk [104–112]. The GST enzymes are active in the detoxification of polycyclic aromatic hydrocarbons in tobacco smoke, halogenated solvents, exposure to TCE or pesticides, and other xenobiotics. However, inconsistency in subgroup findings among studies, small numbers of exposed individuals, and the inability to replicate data suggest that further investigations are needed to clarify these associations.

Asbestos has been associated with elevated renal cancer mortality in two studies, one with insulators and the other with asbestos products workers [110, 113]. However, two extensive meta-analyses of occupational cohort studies of asbestos-exposed workers showed little relation to increased risk for renal cancer [114, 115]. An increased risk of renal cell carcinoma has also been linked to other industrial exposures, including chromium compounds, cadmium, lead, copper sulfate, solvents, benzene, vinyl chloride, pesticides, and herbicides [116–123]. Employment in certain occupations has also been associated with renal cell carcinoma risk, such as printers, aircraft mechanics, farmers, railroad workers, metal workers, mechanics, workers employed in vitamin A and E synthesis, and service station employees [56, 121, 122, 124, 125]. However, none of these occupations or exposures has been conclusively related to risk in epidemiologic studies. Other environmental exposures, such as arsenic, nitrate, and radon in drinking water, also have not been established as risk factors for developing RCC [126–130].

1.3.7 Dietary Factors

Geographic variations in incidence and mortality suggest a role for environmental and dietary factors in the development of RCC. There has not been convincing evidence for a protective role of a diet rich in fruits and vegetables in the development of RCC. A number of case–control studies reporting on associations between intake of fruits and vegetables and RCC risk have given inconclusive results. Although high fruit and vegetable consumption was associated with a decreased risk of RCC in a pooled analysis of several cohort studies, other large prospective cohort studies failed to demonstrate such an association [131–133]. Antioxidants such as vitamins A, C, and E and carotenoids that are common in fruits

and vegetables also have not been consistently linked to renal cell carcinoma risk [134–136].

Dietary habits associated with a western lifestyle, including the consumption of red or processed meat, have been proposed as potential risk factors of RCC. In a meta-analysis of case–control studies, this was associated with increased risk of RCC; however, this association was not confirmed in a pooled analysis of cohort studies [137–139]. A recent report from a cohort study of Swedish women concluded that the risk of renal cell carcinoma was consistently reduced with increasing frequency of fatty fish consumption, but not with lean fish consumption [140].

A study conducted in Sweden detected high levels of acrylamide, a potential carcinogen, in commonly consumed fried and baked foods [141]. However, other epidemiological studies have yielded mixed results suggesting further studies in humans are important given the consumption of food items with elevated acrylamide levels [142, 143].

Moderate alcohol consumption has been inversely associated with renal cell carcinoma risk in a pooled analysis of prospective studies, with an estimated 28 % reduction in risk among those who drank ≥15 g/day, equivalent to slightly more than one alcoholic drink per day [144–146]. This inverse association was observed for all types of alcoholic drinks, including beer, wine, and liquor. In contrast, no association was found with coffee, tea, milk, juice, soda, and water [147]. A potential mechanism by which moderate consumption of alcohol may reduce renal cell carcinoma risk is through improvement in insulin sensitivity, thus lowering the risk of type 2 diabetes, production of insulin-like growth factor-I, and subsequent risk of renal cell carcinoma [148, 149].

Conclusion

Renal cell carcinoma incidence has continued to increase over several decades among all racial groups. This has been in the context of widespread use of diagnostic imaging and increasing prevalence of risk factors leading to the diagnosis of smaller tumors and localized disease. Cigarette smoking, excess body weight leading to increased BMI, and hypertension are established modifiable risk factors of RCC and have likely contributed to the increasing prevalence of RCC in both sexes. The variation in the prevalence of these factors across subpopulations may explain the racial and geographic variation in RCC incidence observed, not only in the United States but worldwide. These risk factors may contribute to as much as 50 % of all RCC cases and are targets for preventative strategies in reducing RCC incidence. The relative contribution of other risk factors such as occupational and environmental exposures, hormonal factors, and dietary considerations are not as clearly elucidated. While only a small proportion of renal cell carcinoma occur within the milieu of familial cancer syndromes, genetic susceptibility and its interplay with environmental exposures play an important role in the etiology and development of sporadic renal cell carcinoma. Genetic polymorphisms may modulate an effect on metabolic activation and detoxification enzymes, which will allow improved analysis and interpretation of exposure associations that are important in the initiation and progression of RCC. The multifactorial nature of RCC requires that further studies are conducted to explain underlying factors that may influence individual risk and to elucidate complex relationships between potential genetic, lifestyle, and environmental elements on cancer development.

Due to the advances in the molecular and genetic biology of renal cell carcinoma, a paradigm shift has occurred in the treatment of patients with advanced renal cell carcinoma. Advances in the molecular genetics of RCC syndromes have allowed earlier genetic testing leading to improvements in detection, surgical interventions, and therapeutic approaches. The development of targeted therapies involving the VEGF and mTOR pathway in renal cell carcinoma has drastically improved the survival and outcomes of patients afflicted with this malignancy.

Clinical Vignette

A 25-year-old Caucasian male with no past medical history presents with gross hematuria. Urinalysis confirms the finding of red blood cells in the urine. A cystoscopic evaluation was unrevealing. A bilateral renal sonogram demonstrated bilateral renal cysts with at least one of the cysts highly suspicious for malignancy due to complexity. The patient's family history is significant for pheochromocytoma in his father and early death from kidney cancer in a paternal aunt. The patient asks his physician whether his clinical presentation is consistent with some form of familial cancer syndrome.

Q: What hereditary cancer syndrome is most likely to be associated with the patient's presentation? What other possible consequences of this syndrome should the physician be aware of?

A: The most likely syndrome involved in this patient's case is that of VHL syndrome (see chapter text for a full description).

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Pathologic Considerations

2

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Contents

Key Points

- There are several major histological subtypes of RCC, including clear cell, papillary, chromophobe, collecting duct, and medullary carcinomas.
- Specific morphological and immunohistochemical features distinguish these RCC subtypes. Careful review by an experienced pathologist will permit definitive diagnosis in the majority of cases.
- Sarcomatoid dedifferentiation can occur in all RCC tumor subtypes. The mechanism of sarcomatoid change is not well characterized but portends a poor prognosis.
- In the past 15 years, a number of new histological subtypes have been identified; including tubulocystic and clear cell papillary RCC. These entities are rare but important for prognostication and for therapeutic decision-making.
- Special studies, including genotyping and fluorescence in situ hybridization, may aid in diagnosing tumors that are difficult to diagnose by conventional means.

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2.1 Introduction

Renal cell carcinoma is a diverse group of malignant tumors of the kidney that arise from the epithelium lining renal tubules. While all these

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_2

carcinomas fall under the rubric of renal cell carcinoma, they have diverse gross appearance, morphologic features, immunohistochemical profile, molecular biology, and natural history. Most important, all of the different RCC types do not respond to the same therapeutic agents.

2.2 Renal Cell Carcinoma Classification

Renal cell carcinoma (RCC) classification has changed in the past 30 years to better embody our understanding of the pathology and molecular biology of these tumors. In 1986, Thoenes et al. published a classification system based on the histopathologic and cytologic features of the tumor cells [1]. This system, sometimes also referred to as the Mainz classification, was used extensively for the next decade. The next milestone was reached as a result of two important workshops on the classification of renal tumors that were held in 1996 and 1997. The first, entitled "Impact of Molecular Genetics on the Classification of Renal Cell Tumours," was held in October 1996 in Heidelberg, Germany. The conclusions of this workshop were referred to as the Heidelberg classification of renal tumors [2]. The second, entitled "Diagnosis and Prognosis of Renal Cell Carcinoma: 1997 Workshop," organized by the American Joint Committee on Cancer (AJCC) and Union Internationale Contre le Cancer (UICC), was held in March 1997 [3]. The current World Health Organization (WHO) classification, which was published in 2004, is based on these two classification systems. Significant changes in the 2004 WHO classification included the change in terminology of conventional RCC to clear cell RCC and the addition of newer RCC types. Since the publication of the WHO classification, more types have been characterized and reported. In 2012, the International Society of Urological Pathologist (ISUP) held a consensus conference on the classification of renal tumors, the results of which are referred to as the ISUP Vancouver Classification of Renal Neoplasia [4]. The ISUP Vancouver classification is built on the foundation of the 2004 WHO classification (Table 2.1) and includes two new categories of tumors (Table 2.2). The first set is "proposed new renal epithelial tumors," which includes RCC types that have been well characterized since the 2004 WHO classification (Table 2.2). The second category is "proposed emerging/provisional tumor entities" (Table 2.2), which has rare tumors that have not been studied in great detail, in part due to the rarity of such tumors. The ISUP

Table 2.1 International Society of Urological Pathology (ISUP) Vancouver modification of WHO (2004) histologic classification of kidney tumors. The most common tumors are listed in this table

Benign tumors
Papillary adenoma
Renal oncocytoma
Metanephric adenoma
Metanephric adenofibroma
Malignant tumors
Clear cell renal cell carcinoma
Multilocular clear cell renal cell neoplasm of low malignant potential
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Carcinoma of the collecting ducts of Bellini
Renal medullary carcinoma
Xp11 translocation carcinomas (MiT family translocation renal cell carcinoma)
Mucinous tubular and spindle cell carcinoma
Carcinoma associated with neuroblastoma
Unclassified renal cell carcinoma

Table 2.2 International Society of Urological Pathology (ISUP) Vancouver classification of kidney tumors. Renal tumors defined since the 2004 WHO classification are listed below

Proposed new renal epithelial tumors
Tubulocystic renal cell carcinoma
MiT family translocation renal cell carcinoma (including t(6;11) renal cell carcinoma)
Clear cell (tubulo)papillary renal cell carcinoma
Hereditary leiomyomatosis renal cell carcinoma syndrome-associated renal cell carcinoma
Acquired cystic disease-associated renal cell carcinoma
Proposed emerging/provisional tumor entities
Thyroid-like follicular renal cell carcinoma
Succinate dehydrogenase (SDH) B deficiency-associated renal cell carcinoma
ALK translocation renal cell carcinoma

Table 2.3 Salient morphologic features of the different renal cell carcinoma types

Renal cell carcinoma (RCC) type	Salient morphologic features	
Clear RCC	Solid, nested, or tubular architecture; thin-walled plexiform vasculature; optically clear cytoplasm	
Papillary RCC	Papillary, tubular, or solid architecture; frequent hemorrhage and necrosis; foamy macrophages and psammomatous microcalcifications	
	Type 1: Cuboidal epithelium with scant cytoplasm and inconspicuous nucleoli	*Type 2*: Columnar pseudostratified epithelium with voluminous cytoplasm and prominent nucleoli
Chromophobe RCC	Solid, tubular, or nested architecture; thick-walled vasculature; clear to eosinophilic cytoplasm with cytoplasmic membrane accentuation; irregular nuclear membrane border with perinuclear clearing	
Collecting duct RCC	Medullary centered with tubulopapillary architecture; inflammatory and desmoplastic stroma; high-grade nuclear atypia with dysplasia of adjacent collecting ducts	
Renal medullary carcinoma	Medullary centered with tubulopapillary, reticular, and microcystic architecture; inflammatory and desmoplastic stroma with prominent neutrophilic infiltrate; high-grade nuclear atypia; sickling of erythrocytes	
Xp11 translocation carcinoma	Papillary and solid architecture with psammomatous microcalcifications; optically clear cytoplasm with eosinophilic inclusions	
Mucinous tubular and spindle cell carcinoma	Tubular and spindle cell pattern with mucinous extracellular matrix; low-grade nuclei	
Clear cell papillary RCC	Cystic tumor with tubulopapillary architecture; clear cytoplasm and apically located, low-grade nuclei	
Tubulocystic RCC	Cystic tumor embedded in fibrous stroma; clear to eosinophilic cytoplasm and prominent nucleoli	
Primary thyroid-like follicular carcinoma	Tubular architecture containing eosinophilic colloid-like material; nuclear grooves and pseudoinclusions	

Vancouver classification will form the basis for the next WHO classification, which will be published in late 2015 or early 2016. Each of the different types of RCC listed in these classification schemes have distinct morphologic features and genetic profiles, which are detailed in the following sections. The salient features of the different RCC types are listed in Table 2.3.

2.2.1 Clear Cell Renal Cell Carcinoma

Clear cell RCC is the most common type, representing 65–75 % of all RCC in most series [5–7]. These often present as a single solid tumor located at the periphery of the renal parenchyma. A bright yellow or light orange color is most characteristic of clear cell RCC. In addition, there may be areas of cyst formation, hemorrhage, and necrosis. The majority of the carcinomas detected today are confined to the kidney; the rest show gross invasion into the perinephric adipose tissue, the renal sinus adipose tissue, or the renal vein. These carcinomas sometimes extend into the inferior vena cava and, rarely, into the right side of the heart. Tumor cells in clear cell RCC are arranged in sheets, nests, or tubules (Fig. 2.1). One of the hallmark histological features is the presence of delicate, interconnecting, sinusoidal type of thin blood vessels, sometimes likened to "chicken wire" (Fig. 2.2). Most tumor cells have optically clear cytoplasm; however, some tumors can have a combination of cells with clear cytoplasm and granular eosinophilic cytoplasm. Clear cell RCCs almost exclusively composed of cells with eosinophilic cytoplasm are rare. The optically clear appearance of these cells is secondary to the lipid and glycogen content in the cell's cytoplasm. Periodic acid Schiff (PAS) histochemical stain, with and without diastase, is the best method for

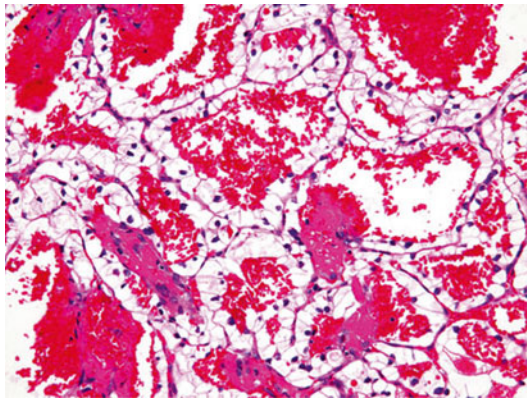

Fig. 2.1 Clear cell renal cell carcinoma, Fuhrman nuclear grade 1, with hemorrhage. H&E stain, 100×

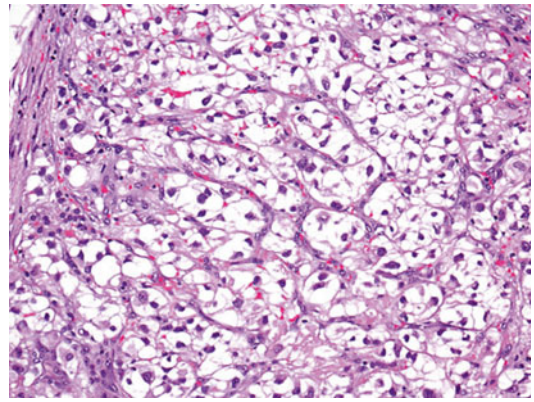

Fig. 2.3 Clear cell renal cell carcinoma, Fuhrman nuclear grade 3, exhibiting clear cells with large nuclei with prominent nucleoli. H&E stain, 100×

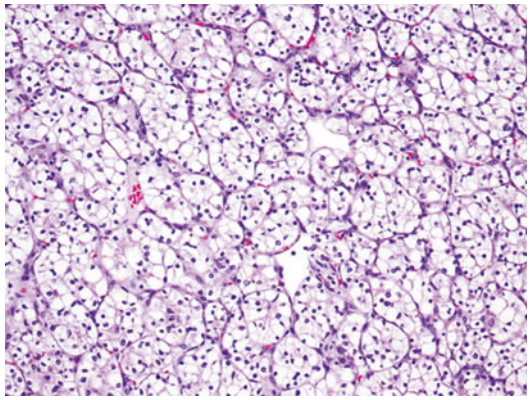

Fig. 2.2 Clear cell renal cell carcinoma, Fuhrman nuclear grade 2, exhibiting typical small nests of clear cells separated by thin sinusoidal blood vessels. H&E stain, 100×

demonstrating the glycogen in the cytoplasm. The diagnosis of clear cell RCC is based on a combination of architectural pattern, vascular pattern, and the cytoplasmic characteristics of the tumor cells, rather than on just the tinctorial properties of the cell cytoplasm (Fig. 2.3).

2.2.2 Papillary Renal Cell Carcinoma

Papillary renal cell carcinoma accounts for about 10–15 % of all RCC. Multifocal and bilateral tumors are more common in this type of RCC. Grossly, these tumors are soft, friable, have a red-brown cut surface, with abundant hemorrhage and necrosis. Some tumors may appear cystic, with a rind of solid tumor at the periphery and most of the tumor cells in the center suspended within hemorrhagic fluid. Papillary RCC is one of the subtypes of RCC most likely to metastasize to regional lymph nodes. In some cases, the regional lymph node metastases form a larger mass than the primary tumor in the kidney. Papilla formation is the typical histologic feature of this RCC. In addition to the papillae, tumor cells may form tubules, tubulopapillary structures, and, rarely, solid nests. Papillary RCC is divided into type 1 and type 2 tumors based on an array of morphologic features [8, 9]. Type 1 papillary RCCs show thin fibrovascular cores that are lined by a single layer of low cuboidal cells that have scant pale cytoplasm and oval low-nuclear-grade nuclei (Fig. 2.4). In contrast, type 2 papillary RCC has tall columnar pseudostratified cells with abundant eosinophilic cytoplasm and high-grade nuclei (Fig. 2.5). As these RCCs are frequently associated with hemorrhage, hemosiderin pigment may be present in the cell cytoplasm, in adjacent histiocytes, and in the stroma. The presence of foamy macrophages within the fibrovascular stalks and laminated calcifications (psammoma bodies) is more commonly present in type 1 tumors. There is evidence accumulating gradually regarding the genetic and clinical differences of these two types [8, 10].

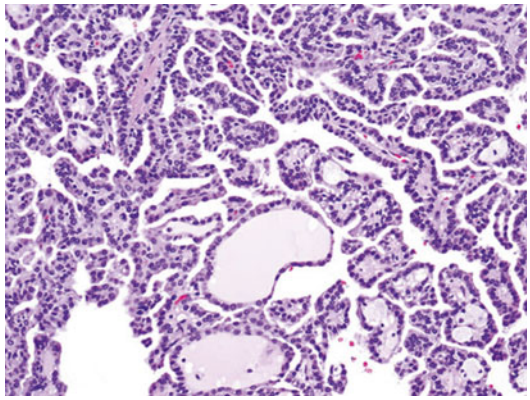

Fig. 2.4 Papillary renal cell carcinoma, type 1. The papillae are lined by cuboidal cells with basophilic cytoplasm and small round nuclei. H&E stain, 100×

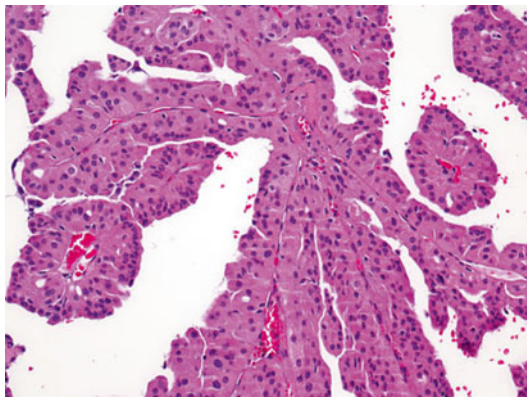

Fig. 2.5 Papillary renal cell carcinoma, type 2. The papillae are lined by tall columnar cells with prominent eosinophilic cytoplasm, large nuclei, and prominent nucleoli. H&E stain, 100×

2.2.3 Chromophobe Renal Cell Carcinoma

Chromophobe RCC accounts for about 5 % of all renal carcinomas. This RCC type was first described in 1985 [11, 12] and exhibits distinctive morphologic, biologic, and ultrastructural features that clearly separate it from the other types. There are two morphological variants, typical or classical chromophobe and the eosinophilic variant. This distinction is based on the physical properties of the cell cytoplasm. Tumor cells are arranged in sheets, broad alveoli, or nests, which are separated by variably spaced thick-walled blood vessels.

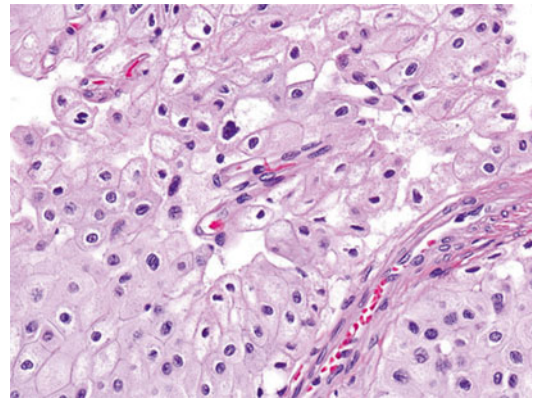

Fig. 2.6 Chromophobe renal cell carcinoma. Tumor cells have pale flocculent cytoplasm, prominent cell membranes, and wrinkled irregular-shaped nuclei. H&E stain, 100×

There are two populations of cells, those with clear cytoplasm and some with eosinophilic cytoplasm. Both cell types are usually present in all tumors, with one cell type predominating. Clear cells have abundant clear cytoplasm with a frothy, flocculent, or bubbly appearance (Fig. 2.6). These cells also have a perinuclear halo due to cytoplasmic organelles being pushed to the periphery forming a rim along the cell membrane. This makes the cell membranes appear thick and prominent with a darker hue than the remainder of the cytoplasm; these cells bear a superficial resemblance to plant cells. The eosinophilic cells tend to be smaller and have finely granular eosinophilic cytoplasm, with a variable degree of perinuclear clearing. The nuclei in both cell types are hyperchromatic and frequently binucleated and have a wrinkled nuclear membrane, resembling koilocytes. Hale's colloidal iron stain is a histochemical stain that is often used for the diagnosis of chromophobe RCC; this stain shows diffuse, reticular staining. At the ultrastructural level, numerous microvesicles are seen in the cell cytoplasm around the nucleus, and the mitochondria have characteristic tubulocystic cristae.

2.2.4 Collecting Duct Carcinoma

Collecting duct carcinoma is rare, accounting for less than 1 % of all RCCs [13–15]. These tumors arise in the medullary region of the kidney.

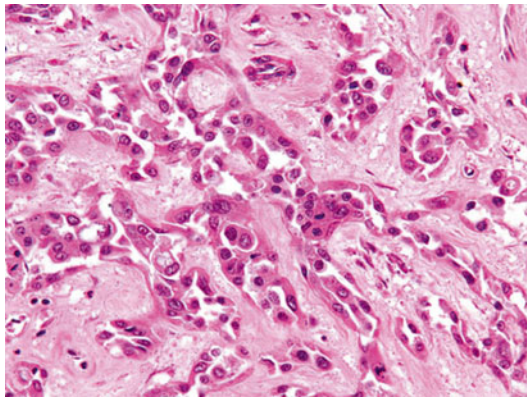

Fig. 2.7 Collecting duct carcinoma of the kidney. The tumor forms glands that are set within dense collagenous stroma. H&E stain, 200×

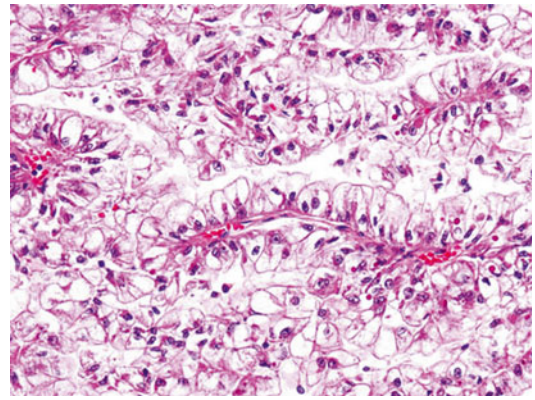

Fig. 2.8 Xp11 translocation carcinoma of the kidney. The tumor forms papillary structures lined by cells with abundant clear cytoplasm. Few cells also have prominent eosinophilic intracytoplasmic inclusions. H&E stain, 100×

Microscopically, three features characterize this renal cancer: a tubulopapillary arrangement of cells, desmoplastic reaction of the stroma, and dysplastic changes in the adjacent collecting ducts. Dilated tubules, glands, and solid areas may also be present (Fig. 2.7). These carcinomas tend to be aggressive, and most patients have a short survival time.

2.2.5 Renal Medullary Carcinoma

Renal medullary carcinoma is a distinctive type of RCC, which arises in the renal medulla, and is associated with the sickle cell trait [16, 17]. This cancer affects young adults, most of whom present with advanced disease and have an aggressive clinical course. These tumors have distinct morphologic features with reticular, microcystic areas, which resemble testicular yolk sac tumor. Foci of mucin and gland-like areas are also present.

2.2.6 Xp11 Translocation Carcinoma

Translocation carcinomas were first described as papillary RCC with specific translocations; but we now know that these are a separate type of RCC. Xp11 translocation carcinomas are more common in children and young adults, with a female predominance. This RCC type comprises approximately one third of all RCCs affecting the pediatric age group [18]. However, we now know that these are not limited to the younger age group, as numerous cases have been reported in the adult population as well. Translocation RCCs often present as locally advanced tumors with extrarenal disease [19, 20]. As the name suggests, these tumors are characterized by translocation of the TFE3 gene, mapping to the Xp11.2 region, with the following partner genes: PRCC gene t(X;1)(p11.2;q21), ASPL gene t(X;17)(p11.2;q25), and PSF gene t(X;1)(p11.2;p34). Another distinct member of this family of tumors is the RCC with fusion of the alpha and TFEB genes t(6;11)(p21;q12). The typical Xp11.2 translocation RCC has a partially papillary architecture, along with solid nests or sheets of tumor cells. The cells have voluminous clear or pale eosinophilic cytoplasm and may have eosinophilic cytoplasmic inclusions (Fig. 2.8). Psammomatous calcifications may also be present. Regardless of the type of fusion event, there is overexpression of the TFE3 gene, which can be detected by immunohistochemical staining for the nuclear TFE3 protein. Of the confirmatory molecular assays, a break-apart TFE3 fluorescence in situ hybridization (FISH) probe is the most practical since tumor cells can be visualized, and, most importantly, no foreknowledge of the fusion partner is required as with RT-PCR approaches. The TFE family of transcription factors activates downstream targets that results in overexpression of specific proteins,

including cathepsin K. Consequently, cathepsin K immunohistochemical stain has been used as a relatively specific immunohistochemical stain for TFE-associated renal carcinomas (both TFE3 and TFEB) as the other common subtypes of RCC, given their different pathogenesis, are cathepsin K negative [21]. The other immunohistochemical stains that may be positive include CD10, cytokeratin, EMA, and vimentin.

2.2.7 Mucinous Tubular and Spindle Cell Carcinoma

Mucinous tubular and spindle cell carcinoma (MTSCC) is a morphologically distinct type of RCC, which superficially resembles papillary RCC. These RCCs are usually small and organ confined; however, sarcomatoid dedifferentiation and metastases have been reported to occur [22–24]. MTSCCs are composed of tubules lined by cuboidal cells that are set within a loose stroma with blue mucin. Foci of bland-appearing spindle cells are also present. The amount of the different components varies from tumor to tumor, with some tumors having more spindle cells than others.

2.2.8 Carcinoma Associated with Neuroblastoma

Pediatric patients who survive childhood neuroblastoma have been reported to have an increased incidence of RCCs. Although only a handful of tumors have been systematically studied, these tumors have a distinctive morphologic appearance. The tumors have varying histologic patterns and have cells with copious eosinophilic or oncocytic cytoplasm [25, 26].

2.2.9 Unclassified Renal Cell Carcinoma

Unclassified RCC is not a distinct type, but rather is a designation for RCC that does not fit into one of the abovementioned categories. As science advances and we develop a better understanding of these rare tumors, other specific types will emerge from the unclassified group. At present, this designation is a sort of "wastebasket" term for tumors that do not neatly fit into any of the usual types listed above. RCCs in this category also include tumors that are composites of the usual types, for example, clear cell RCC and papillary RCC, RCC with extensive necrosis and minimal viable tumor, and RCC with sarcomatoid dedifferentiation where there is a minimal epithelial component that cannot be readily assigned to one of the above categories [3].

2.2.10 Sarcomatoid Dedifferentiation in Renal Cell Carcinoma

The use of the term sarcomatoid RCC was abandoned in the current classification system, as all types of RCC may undergo this transformation. Sarcomatoid dedifferentiation is reported in approximately 2–5 % of all RCC [27], though it is more frequent in advanced disease comprising approximately 20 % of stage IV RCC [28, 29]. The term sarcomatoid dedifferentiation denotes anaplastic transformation of the RCC into a high-grade biphasic tumor that has both malignant elements, that is, carcinomatous and sarcomatous (resembling a mesenchymally derived sarcoma). The carcinomatous component may have any nuclear grade but is usually high grade, usually a Fuhrman nuclear grade 3 (Fig. 2.9a). The sarcomatous component may be undifferentiated, resembling a pleomorphic malignant fibrous histiocytoma (MFH) or an unclassified spindle cell sarcoma (Fig. 2.9b), or rarely may show differentiation (heterologous differentiation) into the bone, cartilage, skeletal muscle, or blood vessels. The differential diagnosis for these tumors also includes primary sarcomas of the kidney, which are rare tumors. The presence of a distinct carcinoma component helps separate primary sarcomas from RCC with sarcomatoid dedifferentiation. Benign spindle cells are sometimes seen in RCC, which need to be differentiated from a true sarcomatoid component. The majority of these tumors present at high stage with poor prognosis. The

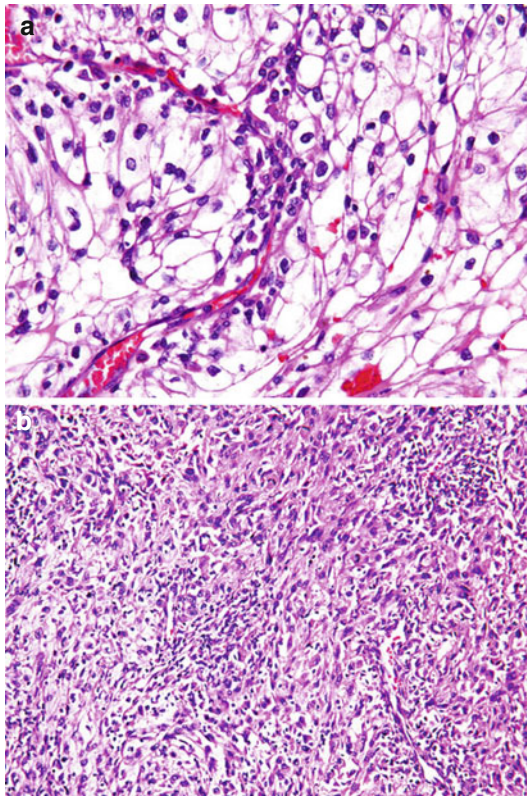

Fig. 2.9 Clear cell renal cell carcinoma with sarcomatoid dedifferentiation. The epithelial component consists of Fuhrman nuclear grade 3 clear cell renal cell carcinoma (**a**). The sarcomatoid component has high-grade spindle cells resembling a soft tissue sarcoma (**b**). H&E stain, 100×

amount of sarcomatoid dedifferentiation (as a percentage of the entire tumor) has historically shown some prognostic value as patients with more than 50 % sarcomatoid dedifferentiation in their tumor tend to do poorly. This point is controversial, however, as RCCs with even a minor sarcomatoid component (5–15 %) have been reported to result in metastasis and cancer-specific death [30, 31], and some recent data suggest no overall correlation between percentage of sarcomatoid elements and cancer-specific mortality [29].

2.2.11 New and Rare Renal Cell Carcinoma Types

Since the publication of the WHO RCC classification, newer, rare subtypes have been described. These are briefly described below and are listed in Table 2.2.

Tubulocystic RCC in the past has been referred to as low-grade collecting duct carcinoma. Tubulocystic carcinoma is a circumscribed, exclusively cystic tumor with interspersed fibrous stroma. Neoplastic cells with clear or eosinophilic cytoplasm and prominent nucleoli line tubules and cysts of varying caliber. Metastatic disease has been documented to occur with these tumors [32, 33].

Clear cell (*tubulo*) *papillary RCC* is another novel type seen in association with ESRD, though it can as well be seen in patients without ESRD. This tumor, arising in a cystic background, is arranged in a predominantly papillary pattern with neoplastic cells containing clear cytoplasm and whose nuclei are low grade (Fuhrman grade 2) and oriented toward the apex of the cell. All clear cell papillary RCCs have been organ confined, with no metastases reported [34, 35].

Hereditary leiomyomatosis RCC (*HLRCC*) *syndrome-associated RCC* is another rare tumor seen in patients with the HLRCC familial syndrome, an autosomal dominant syndrome with germ line mutations of the fumarate hydratase gene. These patients also have uterine and subcutaneous leiomyomas. This tumor superficially resembles type 2 papillary renal cell carcinoma with a tubulopapillary architecture and large cells with eosinophilic cytoplasm. However, the characteristic morphologic feature is the large prominent cherry red nucleolus surrounded by a clear halo. This RCC type is highly aggressive, with most patients presenting with metastatic disease.

Acquired cystic disease-associated RCC, as the name implies, affects patients with end-stage renal disease (ESRD). Although all types of RCC can occur in patients with ESRD, two distinct tumors (acquired cystic disease-associated RCC and clear cell papillary RCC) show an increased predilection in this setting. Acquired cystic disease-associated RCCs are circumscribed tumors with varied architecture showing at least focal cribriform areas. Tumor cells contain abundant eosinophilic cytoplasm with vacuolation and deposition of conspicuous calcium oxalate crystals. Metastasis and one cancer-related death have been reported from this tumor [35].

Primary thyroid-like follicular carcinoma of the kidney shows features reminiscent of follicular

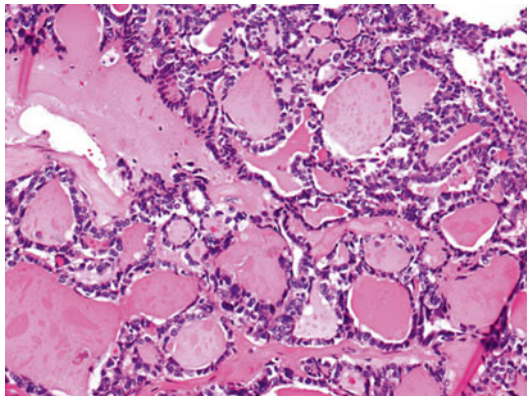

Fig. 2.10 Primary thyroid-like follicular carcinoma of the kidney. The carcinoma shows features reminiscent of follicular thyroid carcinoma with tumor cells forming colloid-filled follicles. H&E stain, 100×

thyroid carcinoma with tumor cells forming colloid-filled follicles (Fig. 2.10). Tumor cells have eosinophilic cytoplasm with nuclear grooves and pseudoinclusions. These tumors stain with RCC markers and are negative for the thyroid stains such as thyroglobulin and TTF-1 [36, 37]. Some patients with these tumors may present with metastatic disease.

Succinate dehydrogenase (*SDH*) *B deficiency-associated RCC* is a rare type of RCC reported in a small number of patients with germ line mutations of the SDHB gene. These tumors have nests of cells with abundant eosinophilic cytoplasm, which may superficially resemble renal oncocytoma. Lack of staining with the SDHB protein immunohistochemical stain has been reported as being sensitive and specific for this rare RCC [4].

ALK translocation RCC is another rare tumor reported in fewer than ten patients [4].

2.3 Ancillary Testing in Renal Cell Carcinoma

The past decade has seen many advances in the treatment of RCC, with a number of the newer targeted therapies being better suited for the treatment of clear cell RCC. In the present era, distinction between the different RCC tumor types is essential for making appropriate therapy decisions. In most cases, the diagnosis can be achieved without the use of ancillary techniques; however, the use of these tests is essential for some RCC types (e.g., translocation RCC) and for confirming the diagnosis of metastatic RCC. Immunohistochemical stains are the most common and widely used technique for this purpose, and the ISUP has provided some guidelines to their judicious use [38]. Electron microscopy was used extensively in the past but now has a limited role. Except for limited use of next-generation sequencing (NGS) at present, newer techniques for molecular diagnosis remain in the research arena but will likely play a more important role in diagnosis of RCC in the coming years. Table 2.4 lists the immunohistochemical profiles and karyotyping by traditional cytogenetic techniques of the different RCC types.

Almost all RCCs stain positive with immunohistochemical stains for cytokeratin cocktail, low-molecular-weight cytokeratin, and epithelial membrane antigen (EMA). However, translocation RCC may lack staining for cytokeratin and EMA in a majority of tumors. PAX-8 stains most RCC and is also useful for identifying metastatic tumors; however, it is not specific for RCC as it is also expressed in thyroid and ovarian tumors. Vimentin, an intermediate filament usually associated with mesenchymal structures, stains most RCC, except for chromophobe RCC. Vimentin is useful for distinguishing the eosinophilic variant of chromophobe RCC from clear cell RCC with predominantly eosinophilic cells. In addition to the abovementioned markers, clear cell RCC typically also stains with RCC antigen (RCC), CD10, CAIX, and CD15 (Leu-M1). Papillary RCCs stain with RCC, CD10, CD15, cytokeratin 7, and alpha-methylacyl-coenzyme A racemase (AMACR). Chromophobe RCCs stain with cytokeratin 7, parvalbumin, and RON proto-oncogene; they lack staining for vimentin, RCC antigen, and CD10. Collecting duct carcinoma, however, has a unique staining pattern, reacting with both low- and high-molecular-weight cytokeratins, peanut agglutinin, *Ulex europaeus* lectin, and epithelial membrane antigen. Mucinous tubular and spindle cell carcinoma stains similar to papillary RCC. Xp11 translocation carcinomas characteristically show staining for cathepsin K and nuclear staining for the TFE3 or TFEB proteins and variably stain with CD10, cytokeratin,

Table 2.4 Immunohistochemical profile and karyotypes (using traditional cytogenetic techniques) of the different renal cell carcinoma types

Renal cell carcinoma (RCC) type	Immunohistochemical profile		Karyotype
	Positive	Negative	
Clear RCC	EMA, VIM, RCC, CD10, CAIX	CK7, AMACR	3p12−, 3p21−, 3p25−, 5q22+
Papillary RCC	EMA, VIM, RCC, CK7 AMACR		+7, +17, −Y
Chromophobe RCC	EMA, CK7, CD117	VIM, CD10, RCC	−1, −2, −6, −10, −13, −17, −21
Collecting duct RCC	Ulex, CK-LMW, CK-HMW, P63, VIM	CD10, RCC	No consistent copy number aberrations
Renal medullary carcinoma	Ulex, CK-LMW, CK-HMW, P63, VIM		−11
Mucinous tubular and spindle cell carcinoma	EMA, VIM, RCC, CK7 AMACR		−1, −4, −6, −8, −9, −13, −14, −15, −22
Xp11 translocation carcinoma	TFE-3, CD10, AMACR	EMA, CK7	t(X;17)(p11.2;q25) t(X;1)(p11.2;q21) t(X;1)(p11.2;p34)
Clear cell papillary RCC	CK7, EMA	AMACR, CD10	No gains/losses
Tubulocystic RCC	CK7, CD10, AMACR	CK-HMW	+17
Primary thyroid-like follicular carcinoma	CK7, VIM	TTF-1, thyroglobulin, RCC	No consistent copy number aberrations

AMACR alpha-methylacyl-CoA racemase, *CAIX* carbonic anhydrase IX, *CK* cytokeratin, *CK-LMW* low-molecular-weight cytokeratin, *CK-HMW* high-molecular-weight cytokeratin, *EMA* epithelial membrane antigen, *RCC* renal cell carcinoma antigen, *TTF-1* thyroid transcription factor 1, *Ulex Ulex europaeus* lectin, *VIM* vimentin

EMA, and vimentin. Acquired cystic disease-associated RCCs typically stain positive for AMACR and negative for cytokeratin 7 and EMA. Clear cell papillary RCCs, by contrast, characteristically stain positive for cytokeratin 7 and CAIX (with a distinct "cuplike" pattern of staining) and are negative for CD10 and AMACR. Tubulocystic carcinomas show consistent immunoreactivity for CD10, cytokeratin 7, and AMACR. Thyroid follicular-like carcinomas are negative for most RCC-associated markers but, importantly, are also negative for the thyroid transcription factor (TTF1) and thyroglobulin.

Ultrastructurally, the cells of clear cell RCC exhibit a brush border, tend to form microlumina, and have a basal lamina that separates groups of cells from each other. Abundant glycogen and lipid are present in the cytoplasm. Chromophobe RCC has characteristic microvesicles, which are probably derived from the endoplasmic reticulum or from mitochondria. Mitochondria also impart the characteristic granularity to the cytoplasm seen by light microscopy.

The molecular biology of RCC is elaborated on elsewhere in this text but is briefly described here as it may help in classifying these tumors. Karyotyping using traditional cytogenetics was one of the first and is likely still the most commonly used method to aid in the differential diagnosis of RCC. Since the identification of some of the characteristic genetic abnormalities, FISH has also been used to detect specific losses or gains of chromosome segments. Newer techniques for DNA sequencing and RNA sequencing have also been shown to successfully distinguish between the common types. However, while all these techniques are valuable, their adoption in the clinical setting has been slow considering the cost and technical challenges. Sporadic clear cell RCC typically (in approximately 80–90 %) shows loss of genetic material from the short arm of chromosome 3. Mutations within the VHL gene region and inactivation of this gene by hypermethylation are the most common genetic abnormalities. Sporadic papillary RCC is characterized by trisomies, especially of chromosomes 7 and 17, and loss of the

Y chromosome. Other chromosomes that may be involved include 3, 9, 11, 12, 16, and 20; some of these additional abnormalities are speculated to lead to progression to a more aggressive phenotype. Translocation between chromosomes X and 1 has also been reported more commonly in children; however, these tumors may actually represent translocation RCC. Familial cases of papillary RCC show germ line mutations of the MET proto-oncogene. Chromophobe RCCs are characterized by combined losses of multiple whole chromosomes including 1, 2, 6, 10, 12, 13, 14, 15, and 17. Polysomy of chromosome 7; trisomy 12, 16, and 19; telomeric associations; and structural abnormalities of 11q have also been reported in these cancers. Another important finding in chromophobe RCC is abnormalities of mitochondrial DNA, a feature not seen in the other subtypes. Collecting duct carcinoma does not have any distinct genetic alterations. As mentioned above, the different translocation carcinomas show specific genetic translocations. Among the newly described renal carcinomas, the clear cell and papillary carcinoma is notable for its absence of any DNA copy number alterations or VHL mutation or hypermethylation that is characteristic of the more well-established renal cancers [39].

2.4 Grading of Renal Cell Carcinoma

Renal cell carcinomas are graded according to Fuhrman nuclear grading system [40], which is divided into four grades based on the nuclear size, nuclear anaplasia, and nucleolar size (Table 2.5). A nuclear grade is assigned based on the highest grade within the entire tumor and is not dependent on the nuclear grade that is predominant. It is evaluated at 100× and 400× magnification using a light microscope. Clinical utility of the Fuhrman nuclear grading system has only been proven in clear cell RCC [41] and not in the other types of RCC. The 2012 ISUP consensus meeting formalized the notion that nucleolar grading is emerging as a simpler alternative to Fuhrman grading and one that correlates better with prognosis [42, 43]. Both the Fuhrman and ISUP grading schema are appropriate for grading clear cell RCC and papillary RCC, but not for chromophobe RCC.

Table 2.5 Fuhrman nuclear grading for renal cell carcinoma

Grade	Nucleus	Nucleolus
1	Small (10-μm diameter), round, uniform, resembling nucleus of mature lymphocyte	Inconspicuous or absent nucleoli (viewed at 400× magnification)
2	Larger nuclei (15-μm diameter), with slight nuclear irregularity	Small nucleoli (only visible at 400× magnification)
3	Large nuclei (20-μm diameter), with obvious nuclear irregularity	Large, prominent nucleoli (visible at 100× magnification)
4	Same as grade 3 but more bizarre with multilobation and large clumps of chromatin	Large, prominent nucleoli (visible at 100× magnification)

2.5 Pathologic Staging of Renal Cell Carcinoma

The AJCC tumor, nodes, and metastasis (TNM) system is the most widely used system for staging RCC (Table 2.6). The older system known as Robson's staging is no longer used. As with other organs, the TNM staging system is based on the size and extent of invasion by the tumor. The organ-confined tumors are low stage (pT1 and pT2), which are then further divided based on the size. The higher-stage tumors (pT3 and pT4) extend beyond the confines of the kidney. One of the important changes to the staging system occurred in the 2002 TNM staging system, which was the inclusion of renal sinus invasion into the pT3a category. The recognition of this invasion is dependent on pathologic sampling of the tumor in the renal hilar region. In the 2010 TNM system, the most significant changes are related to direct invasion of the ipsilateral adrenal gland by RCC (changed from pT3a to pT4), invasion of the renal vein (changed from pT3b to pT3a), invasion into the inferior vena cava (changed from pT3c to pT3b), and changes in the N stage (simplified to N0 and N1).

Table 2.6 2010 TNM staging system for renal cell carcinoma

Primary tumor (*T*)	
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pT1	Tumor 7.0 cm or less in greatest dimension, limited to the kidney
pT1a	Tumor 4 cm or less in greatest dimension, limited to the kidney
pT1b	Tumor more than 4 cm but not more than 7 cm in greatest dimension, limited to the kidney
pT2	Tumor more than 7.0 cm in greatest dimension, limited to the kidney
pT2a	Tumor more than 7 cm but less than or equal to 10 cm in greatest dimension, limited to the kidney
pT2b	Tumor more than 10 cm, limited to the kidney
pT3	Tumor extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota's fascia
pT3a	Tumor grossly extends into the renal vein or its segmental (muscle containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond Gerota's fascia
pT3b	Tumor grossly extends into the vena cava below the diaphragm
pT3c	Tumor grossly extends into vena cava above diaphragm or invades the wall of the vena cava
pT4	Tumor invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)
Distant metastasis (M)	
MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage groupings			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1–T3	N1	M0
Stage IV	T4	N0—N1	M0
	Any T	N2	M0
	Any T	Any N	M1

Clinical Vignette

A 30-year-old man was referred to a tertiary care medical center for a second opinion. Four months earlier, he developed gross, painless hematuria, and workup revealed a right-sided renal parenchymal tumor. Imaging also revealed some suspicious retroperitoneal adenopathy. He underwent a radical nephrectomy, and initial pathology review revealed an 8 cm tumor, nuclear grade 3, with extrarenal extension, and three out of six lymph nodes positive. Histological evaluation demonstrated predominant clear cell features but also showed a subset of papillary characteristics. The patient was diagnosed as having "predominant clear cell features." Follow-up CT scan of the chest, abdomen, and pelvis 2 months later revealed scattered pulmonary nodules and new and progressive retroperitoneal and mediastinal adenopathy. The patient was referred for consideration of a clinical trial. Pathological rereview revealed a tumor with solid sheets of clear tumor cells intercalated with a partially papillary architecture. Immunohistochemical stains were

positive for CD10, cytokeratin, EMA, and vimentin. The tumor also showed strong staining for TFE3 and cathepsin K. FISH confirmed an Xp11.2 translocation.

This case illustrates the clinical and pathologic characteristics seen in patients with translocation carcinoma. This histology is typically seen in a younger patient demographic and shows a mixture of clear and papillary features. TFE3 staining is not 100 % sensitive or specific but can guide the diagnosis of this rare but characteristic tumor subtype.

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Molecular Biology of Kidney Cancer

3

William G. Kaelin Jr.

Contents

Key Points

- von Hippel-Lindau (VHL) gene mutation is the hallmark of clear cell renal cell carcinoma (ccRCC).
- Disruption of VHL results in upregulation of a number of hypoxia-inducible factor (HIF)-regulated genes involved in angiogenesis; these gene products are responsible for the vascular nature of VHL-related lesions.
- VHL has a number of non-HIF-related functions whose loss likely contributes to the development of the cancer phenotype.
- Therapies targeting the vascular endothelial growth factor (VEGF) axis have arisen directly from our understanding of the molecular biology of VHL.
- A number of other potential VHL- and HIF-related targets are being investigated, including cell-matrix-interacting proteins, other growth factors, and canonical signaling pathways.
- The recent discovery of additional mutations in RCC affecting histone function, including BAP1, PBRM1, and SETD2, provides new research avenues for therapy development.
- A better understanding of the molecular biology of immune cell response has also provided exciting new agents, including anti-CTLA-4 and anti-PD1 antibodies.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_3

3.1 Introduction

Kidney cancer is one of the ten most common cancers in the United States. Approximately 75 % of kidney cancers are clear cell renal carcinomas, and most clear cell renal carcinomas are linked to inactivation of the von Hippel-Lindau tumor suppressor gene (*VHL*). Studies of the *VHL* gene product, pVHL, revealed that it participates in the oxygen-dependent degradation of the HIF (hypoxia-inducible factor) transcription factor. HIF is a master regulator of genes, such as vascular endothelial growth factor (VEGF), that participate in adaptation to hypoxia. The mTOR kinase also affects HIF protein and may also participate in signaling downstream of VEGF. Collectively these discoveries provided a conceptual framework for the testing, and eventual approval, of VEGF inhibitors and mTOR inhibitors for the treatment of kidney cancer. This chapter will review the molecular biology of kidney cancer, focusing on the role of pVHL in clear cell renal carcinoma.

3.2 The von Hippel-Lindau Tumor Suppressor Gene

von Hippel-Lindau disease is characterized by an increased risk of clear cell renal carcinoma; hemangioblastomas of the retina, spinal cord, and cerebellum; and pheochromocytoma [1]. Pioneering studies by Bert Zbar, Marston Linehan, and Eamon Maher led to the identification of the gene that, when mutated in the germline, causes this disease (*VHL*) [2]. The human *VHL* gene is located on 3p25 and contains three exons. VHL orthologs have now been identified in a wide variety of metazoan species. Individuals with VHL disease have inherited a defective *VHL* allele from one of their parents or, less commonly, have a de novo *VHL* mutation. The development of tumors in VHL disease is linked to inactivation of the remaining wild-type *VHL* allele in a susceptible cell. As such, VHL conforms to the Knudson 2-hit model. In keeping with the increased risk of clear cell renal carcinoma in VHL patients, biallelic *VHL* inactivation, due to somatic *VHL* mutations or *VHL* hypermethylation, is also very common in sporadic (nonhereditary) clear cell renal carcinomas [3]. In many early studies, *VHL* mutations were documented in about 50 % of sporadic clear cell renal carcinomas, with another 5–20 % of tumors exhibiting *VHL* hypermethylation, which inhibits transcription of the *VHL* gene. More recent studies, using newer sequencing methods, suggest that the frequency of *VHL* mutations in clear cell renal carcinoma is actually much higher [4, 5]. This would explain why the vast majority of clear cell renal carcinomas have molecular signatures suggestive of *VHL* inactivation (see also below) [6].

One can infer the evolutionary history of a given tumor by determining the frequency of specific mutant alleles (and hence subclones) within that tumor by next-generation sequencing. Such studies confirm that biallelic *VHL* inactivation is an early "truncal" event in clear cell renal carcinoma but is not sufficient to cause this disease [7–10].

3.3 The VHL Tumor Suppressor Protein

The *VHL* mRNA is actually translated into two different proteins by virtue of alternative, in-frame, translation initiation codons [11–13]. The long form contains 213 amino acids. The short form is missing the first 53 amino acid residues. In most, but not all, biological assays, the short form and long form behave similarly. Moreover, virtually all of the *VHL* mutations identified to date affect both the long and the short forms of the protein. Therefore, "pVHL" will be used throughout this chapter when referring to the two protein isoforms generically. pVHL resides primarily in the cytoplasm [14, 15] but shuttles dynamically to and from the nucleus [16, 17]. Some pVHL can also be detected in mitochondria [18] and in association with the endoplasmic reticulum [19]. Restoration of pVHL function in *VHL*−/− clear cell renal carcinomas suppresses their ability to form tumors in vivo but not their ability to proliferate on plastic dishes under standard cell culture conditions [15, 20]. pVHL does, however, inhibit proliferation when cells are grown on specific extracellular matrices, at high confluence, or as three-dimensional spheroids [21–25].

VHL-associated neoplasms, including clear cell renal carcinomas, are often highly angiogenic and occasionally cause the excessive production

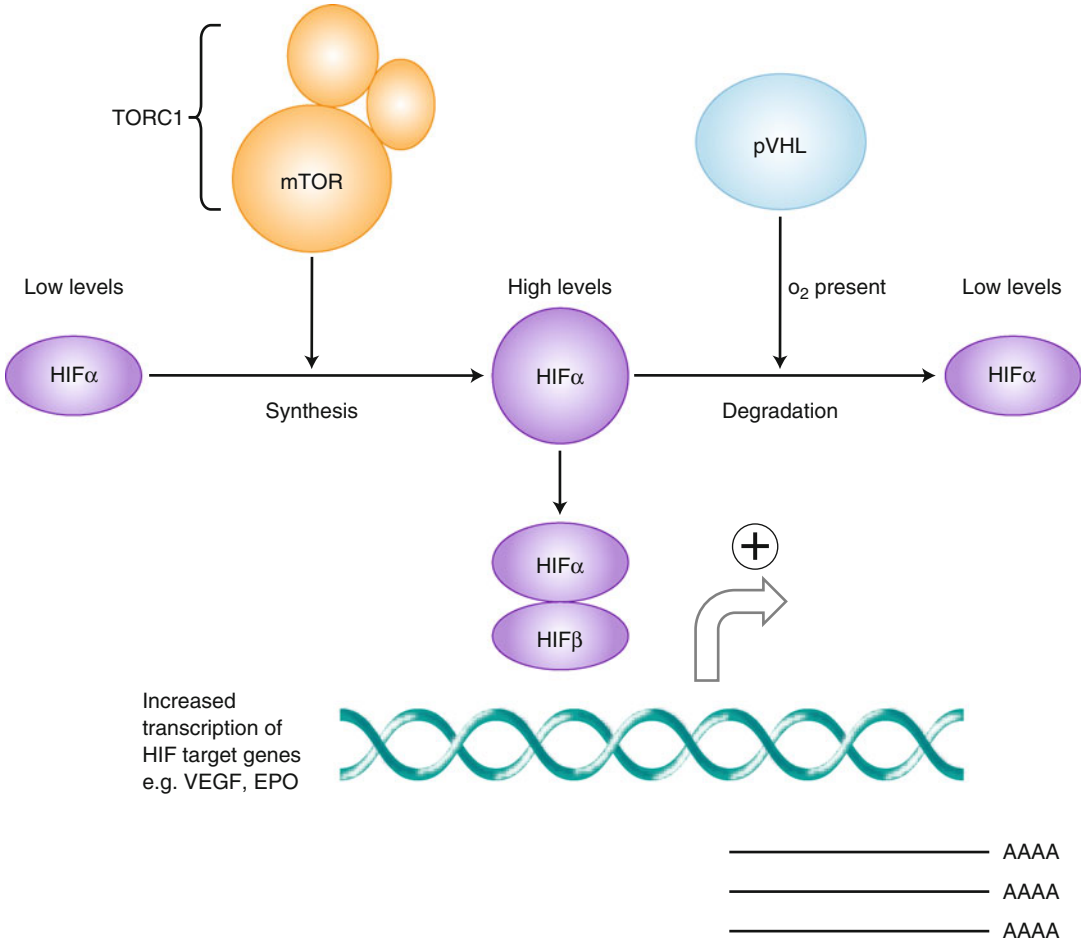

Fig. 3.1 Control of HIF activity. Steady-state levels of HIFα are controlled by its rate of synthesis and degradation. The former is regulated by the TORC1 complex, which contains the mTOR kinase. This is especially true for HIF1α. The rate of degradation is under the control of pVHL. When oxygen is present, HIFα becomes prolyl hydroxylated, which marks it for polyubiquitylation by pVHL and subsequent proteasomal degradation. HIFα can dimerize with its partner protein, HIFβ (also called ARNT), and transcriptionally activate genes such as *VEGF* and *EPO*

of red blood cells (polycythemia). The former is linked, at least partly, to overproduction of VEGF and the latter to secretion of erythropoietin. These clinical features provided important clues with respect to the biochemical functions of pVHL. In particular, pVHL suppresses the production of hypoxia-inducible mRNAs, including the mRNAs for VEGF and erythropoietin, under normal oxygen conditions [20, 26–29]. Consequently, overproduction of such mRNAs, and the proteins they encode, is a hallmark of pVHL-defective tumors.

Mechanistically, pVHL is part of a multiprotein complex that also contains elongin B, elongin C, Cul2, and Rbx1 [30–35]. This complex possesses ubiquitin ligase activity [36–41] and can polyubiquitylate specific substrates, which are then earmarked for destruction by the proteasome. pVHL serves as the substrate recognition component of this ubiquitin ligase complex. The best-documented target of the pVHL ubiquitin ligase is the HIF (hypoxia-inducible factor) transcription factor, which is a heterodimer consisting of an unstable alpha subunit and a stable beta subunit. In the presence of oxygen pVHL binds directly to the HIF alpha subunit and targets it for polyubiquitylation and subsequent proteasomal degradation [28, 38–42] (Fig. 3.1). Under low-oxygen conditions, or in cells lacking functional pVHL, HIFα accumulates

and binds to HIFβ. The HIF heterodimer binds to specific DNA sequences called hypoxia response elements (HREs) in hypoxia-responsive genes such as VEGF and EPO and increases their rate of transcription (Fig. 3.1).

The interaction between pVHL and HIFα requires oxygen because HIFα must be hydroxylated on one (or both) of two conserved prolyl residues in order to be recognized by pVHL [43–47]. Prolyl hydroxylation of HIFα is catalyzed by members of the EglN family [48–50], which are oxygen-dependent enzymes that serve as cellular oxygen sensors [51]. pVHL contains mutational hotspots called the alpha domain and the beta domain. The alpha domain binds directly to elongin C [30, 52], which recruits the remaining members of the ubitquitin ligase complex, and the beta domain binds directly to hydroxylated HIFα [38, 53, 54].

3.3.1 Role of HIF in Clear Cell Renal Carcinoma

There are three HIFα family members called HIF1α, HIF2α, and HIF3α. Deregulation of HIFα, in particular HIF2α, appears to be a driving force in pVHL-defective kidney cancer. For example, the risk of renal carcinoma linked to different *VHL* mutations correlates with the degree to which those mutations deregulate HIF [55–57]. *VHL*−/− renal carcinoma cells frequently silence the expression of FBP1, which is an other endogenous inhibitor of HIF activity [58].

pVHL-defective clear cell renal carcinomas overproduce HIF2α but, in some cases, fail to produce HIF1α [28, 42, 59, 60]. Production of a non-hydroxylatable version of HIF2α, but not HIF1α, can override the tumor suppressor activity of pVHL in preclinical models [61, 62]. Similarly, exogenous overexpression of HIF2α, but not HIF1α, promotes tumor formation by *VHL*−/− renal cancer cells [63, 64]. Moreover, downregulation of HIF2α, but not HIF1α, is sufficient to suppress tumor formation by pVHL-defective clear cell renal carcinomas [65, 66]. The appearance of HIF2α in premalignant renal lesions in patients with VHL disease heralds malignant transformation [67, 68], and a human single nucleotide polymorphism (SNP) linked to HIF2α on chromosome 2p21 has been associated with the risk of developing clear cell renal carcinomas [69]. Finally, much of the pathology observed after *VHL* inactivation in genetically engineered mouse models can be linked to the inappropriate accumulation of HIF2α [68, 70–75]. It should be noted that *VHL* inactivation, but not bona fide hypoxia, is sufficient to induce HIF2α in mouse renal tubular epithelial cells and cause renal cyst formation [68, 72, 76]. Neither *VHL* inactivation nor increased HIF2α activity, however, is sufficient to cause clear cell renal carcinoma in genetically engineered mouse models [68, 72, 76, 77]. This presumably reflects the need for cooperating genetic events (see below) and perhaps species differences.

As noted above, some clear cell renal carcinoma cell lines and tumors produce low, or undetectable, amounts of HIF1α. Indeed, some *VHL*−/− clear cell renal carcinoma lines harbor homozygous mutations of the *HIF1α* locus [60]. Reintroduction of wild-type HIF1α into such lines suppresses their proliferation in cell culture and in nude mice xenograft studies [60, 63, 64]. Conversely, downregulation of HIF1α in HIF1α-proficient *VHL*−/− clear cell renal carcinoma lines enhances their proliferation in cell culture and in xenograft assays [59, 60]. Interestingly, *HIF1α* resides on chromosome 14q, which is frequently deleted in clear cell renal carcinomas (together with chromosome 3p loss and chromosome 5q amplification) [60]. Clear cell renal carcinomas with chromosome 14q deletions have gene expression signatures consistent with decreased HIF1α activity [60, 78]. In some *VHL*−/− clear cell carcinomas that express both HIF1α and HIF2α, the ratio of HIF2α to HIF1α is enhanced by loss of specific microRNAs miR-30c-2-3p and miR-30A-3p that normally serve to repress HIF2α [79]. Finally, loss-of-function intragenic *HIF1α* mutations have occasionally been identified in *VHL*−/− clear cell renal carcinomas [60, 80–82]. Collectively, these findings suggest that HIF1α, in contrast to HIF2α, acts as a tumor suppressor in *VHL*−/− clear cell renal carcinoma.

In apparent disagreement with this contention, expression of a stabilized version of HIF1α, but not a stabilized version of HIF2α, in the proximal renal tubular epithelial cells of mice caused renal cell dysplasia, including evidence of increased

proliferation, increased DNA damage, and clear cell histological changes [83, 84]. Similarly, ablation of *VHL* in primarily mouse collecting ducts caused hyperplastic changes that could be reversed by simultaneous inactivation of HIF1α [85]. Finally, it has also been shown that silencing HIF1α inhibits, rather than augments, tumor growth by human *VHL*+/+ renal carcinoma growth [86].

There are a number of caveats to these studies, however. For example, the cell of origin for *VHL*−/− clear cell renal carcinoma is still debated but likely involves a distal tubular epithelial cell that is permissive for HIF2α accumulation and the expression of specific HIF2α target genes (e.g. cyclin D1) following pVHL loss [67, 68, 87]. In this regard, forced expression of a stabilized version of HIF2α in the murine proximal renal tubule did not recapitulate the induction of HIF targets seen in *VHL*−/− clear cell renal carcinoma [83], perhaps because the wrong cell type was targeted. The genetically engineered mouse studies might also be confounded by biological differences between mice and men, as has been observed with many other cancer genes. Finally, the apparent dependence of human *VHL*+/+ renal carcinomas on HIF1α for tumor growth does not preclude a tumor suppressor role for HIF1α in *VHL*−/− renal carcinomas, especially bearing in mind potential differences in cell of origin and cooperating genetic events.

There are a number of quantitative and qualitative differences between HIF1α and HIF2α that could account for their seemingly antagonistic effects in *VHL*−/− clear cell renal carcinoma. These differences likely reflect the fact that some HIF target genes are preferentially activated by specific HIFα family members as well as by the existence of non-canonical HIF functions that are unique to specific HIFα proteins. HIF2α cooperates with c-Myc to promote the proliferation of *VHL*−/− clear cell renal carcinoma cells, while HIF1α is capable of inhibiting c-Myc [88–91]. Both HIF1α and HIF2α can induce REDD1 and thereby suppress the activity of the TORC1 complex, which contains mTOR, and Cap-dependent translation [92–95]. HIF2α, however, and not HIF1α, can also stimulate translation. HIF2α transcriptionally induces the amino acid transporter *SLC7A5* and thereby increases intracellular amino acid availability, which activates TORC1 [96]. In addition, HIF2α forms a complex with RBM4 and eIF4E that promotes Cap-dependent translation in cells with depressed TORC1 activity [97]. HIF1α and HIF2α also appear to differentially regulate p53 and the DNA damage response [59, 63, 98, 99].

pVHL has a number of other functions that, although incompletely understood biochemically, appear to be a least partly HIF-independent. These include a role in the maintenance of a specialized structure called the primary cilium on the cell surface that serves as a mechanosensor [76, 100–103], possibly by virtue of pVHL's role in stabilization of microtubules [104–106]. Interestingly, a number of diseases characterized by visceral cyst formation, including VHL disease, are caused by mutations that disrupt the primary cilium [107, 108]. pVHL also suppresses autophagy via both HIF-independent and HIF-dependent pathways, perhaps contributing to the increased autophagy seen in clear cell renal carcinomas [109, 110]. In addition, pVHL plays roles in extracellular matrix formation by fibronectin [111–114], epithelial-epithelial contacts [115, 116], NFκB signaling [117–120], control of atypical PKC activity [121–125], Rpb1 expression and activity [126–128], receptor internalization [129–131], and mRNA turnover [20, 26, 132–135]. It is possible that these other functions also contribute to tumor suppression by pVHL.

3.3.2 Cooperating Events

It is clear that pVHL loss is an important, but not sufficient, step in renal carcinogenesis. This is most clearly demonstrated by studies of the natural history of von Hippel-Lindau disease. Patients with von Hippel-Lindau disease can develop hundreds of premalignant renal cysts, very few of which will go on to become clear cell renal carcinomas [67, 136] (Fig. 3.2). This bottleneck presumably reflects the requirement for additional genetic events, occurring stochastically, to fully transform renal epithelial cells. Indeed, a number

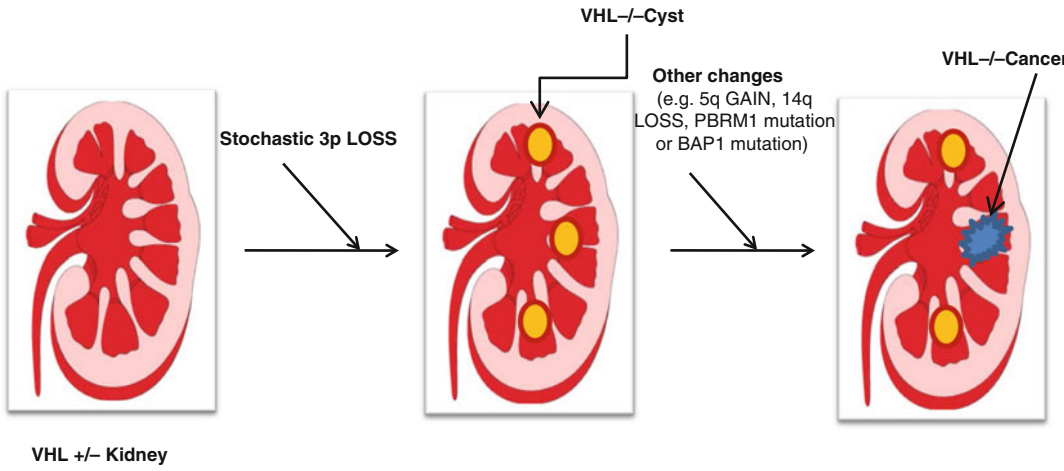

Fig 3.2 Development of renal cell carcinoma in VHL patients. VHL patients are *VHL* heterozygotes, having one normal *VHL* allele and one defective allele. Loss of the remaining normal allele in kidney cells, occurring stochastically, leads to the development of preneoplastic renal cysts. A minority of such cysts will ultimately accumulate additional genetic changes and become clear cell renal carcinomas. Such genetic changes include gain of 5q, loss of 14q, as well as intragenic mutations of specific genes such as *PBRM1* or *BAP1*

of nonrandom genomic abnormalities have been described in clear cell renal carcinoma including, most notably, 5q amplification and 14q loss [6, 137–143] (Fig. 3.2). The triad of 3p loss, 14q loss, and 5q gain is a signature of clear cell renal carcinoma, and some clear cell renal carcinomas have unbalanced translocations involving 3p and 5q that result in loss of 3p and gain of 5q sequences [60, 144–150].

Loss of chromosome 3p, which harbors the *VHL* tumor suppressor gene, is the most common genetic event in kidney cancer. Chromosome 3p has been suspected for many years, however, to contain at least one additional kidney cancer suppressor gene. Indeed, it is now clear that 3p harbors several renal cancer suppressor genes other than *VHL* including *PBRM1*, which encodes the BAF180 chromatin-associated protein; *SETD2*, which encodes a histone H3 lysine 36 methyltransferase; and *BAP1*, which encodes a ubiquitin hydrolase [82, 151–156] (Fig. 3.3). *PBRM1* is, after *VHL*, the most frequently mutated gene in clear cell renal carcinoma. *PBRM1* and *BAP1* mutations are largely mutually exclusive and appear to define clinically distinct subgroups of renal cancers [152, 157, 158].

As described above, *HIF1α* is a likely target of the 14q deletions in *VHL–/–* clear cell renal carcinomas. These deletions are very large, however, suggesting there are additional renal cancer suppressor genes located at 14q. It should also be noted that most 14q deleted *VHL–/–* clear cell renal tumors (in contrast to cell lines) appear to retain a wild-type *HIF1α* allele [60]. This suggests that *HIF1α* is a haploinsufficient clear cell renal carcinoma suppressor and that loss of the remaining allele is associated with tumor progression in vivo or establishment of cell lines ex vivo.

SQSTM1, encoding p62, appears to be one of the renal carcinoma 5q oncogenes [159]. Increased expression of p62 promotes the growth of *VHL–/–* renal carcinoma cells in cell culture and tumor xenograft assays and increases their resistance to redox stress [159]. p62 plays important roles in autophagy and also signals to renal carcinoma relevant proteins including NRF2, NFκB, and mTOR [160–162].

Sequencing of kidney cancer genomes has identified additional genes that, when mutated, contribute to renal carcinogenesis including several more genes linked to chromatin regulation such as *JARID1C* (also known as *KDM5C*), which encodes a histone H3 lysine 4 demethylase; *UTX* (KMD6A), which encodes a histone H3 lysine 27 demethylase; and *ARID1A*,

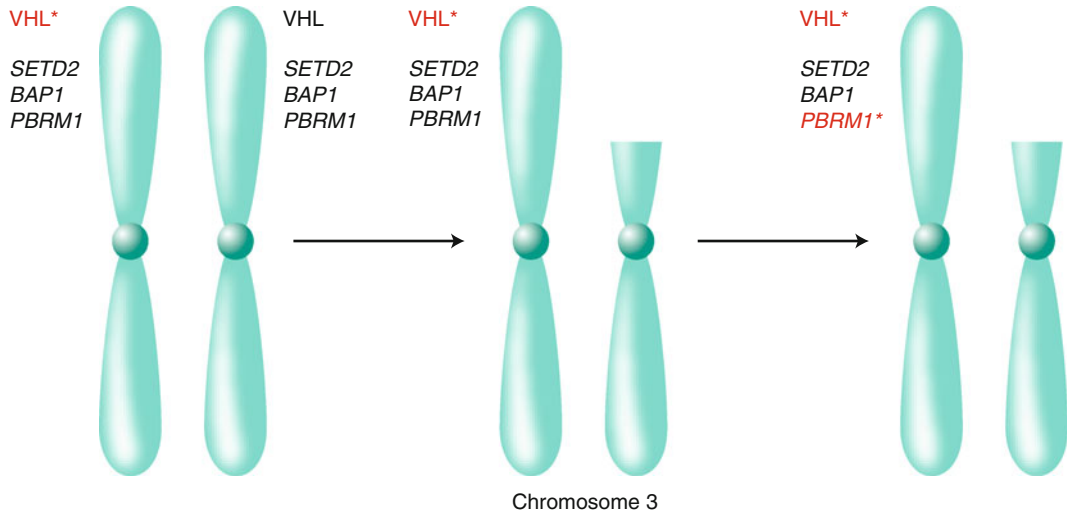

Fig 3.3 Chromosome 3p harbors multiple renal cancer suppressors. Biallelic inactivation of the *VHL* tumor suppressor gene on chromosome 3p, usually as the result of intragenic mutation (indicated by the *asterisk*) followed by loss of the remaining wild-type allele because of a gross 3p deletion, is a critical early event in most clear cell renal carcinomas. The 3p deletions in clear cell renal carcinoma typically span *VHL*, on 3p25, as well as the additional renal cancer suppressors *SETD2*, *BAP1*, and *PBRM1* on 3p21. As a result, subsequent intragenic mutations of these genes deprive renal cells of their wild-type protein products (for illustrative purposes *PBRM1* is shown to be mutated)

a component of a chromatin remodeling complex [82, 151–156, 163]. Notably, many histone demethylase genes are themselves transcriptionally induced by HIF [164–169]. It is possible that their inappropriate expression pursuant to *VHL* loss alters chromatin structure and creates the selection pressure to mutate specific chromatin regulators.

Genes linked to the mTOR pathway including *PIK3CA*, *PTEN*, *TSC1*, *TSC2*, and *MTOR* itself are occasionally mutated in clear cell renal carcinomas [7, 82, 152, 155, 156]. Preliminary data suggest that such mutations identify a subset of renal cell carcinoma patients more likely to derive significant benefit from TORC1 inhibitors [170].

The *NFE2L* gene, encoding NRF2, and the NRF2-negative regulator *KEAP1* are occasionally mutated in clear cell renal carcinoma [82, 159]. Such mutations appear to be mutually exclusive with higher level *SQSTM1* amplification [159]. Genes involved in the response to DNA damage, including *p53*, *MDM4*, and *ATM*, are also occasionally mutated in clear cell renal carcinoma [7, 82, 155, 156]. *p53* loss cooperates with *Vhl* loss in mouse models to promote renal carcinogenesis [171].

3.3.3 Treatment of Renal Cell Carcinoma: HIF Antagonists

The preclinical data outlined above suggest that drugs that inhibit HIF, and particularly HIF2α, would have antitumor activity in kidney cancer. Unfortunately, DNA-binding transcription factors, with the exception of the steroid hormone receptors, have historically been difficult to target with drug-like small molecules. Nonetheless, a number of approaches to targeting HIF are being explored in the laboratory, including the use of DNA-binding polyamides [172–174] and short interfering RNAs [175]. Moreover, HIF2α, but not HIF1α, has a potentially druggable pocket, and lead compounds have been identified that can inhibit HIF2α in biochemical, cell-based, and animal models [176–178].

3.3.4 Treatment of Renal Cell Carcinoma: mTOR Inhibitors

mTOR participates in two complexes, called TORC1 and TORC2 [179]. The former can be inhibited with rapamycin-like drugs. Two such drugs, temsirolimus and everolimus, have been FDA approved for the treatment of renal cell carcinoma based on positive randomized clinical trial data [180, 181]. In theory the activity of these agents reflects direct effects on tumor cells, including modulation of HIF [182], and effects downstream of VEGF signaling in endothelial cells (see below). In preclinical models, *VHL*−/− renal carcinoma lines are more sensitive to rapamycin than are their pVHL-proficient counterparts [183]. As noted above, preliminary data suggest that concurrent mutations of the PI3K-MTOR pathway are enriched among renal carcinoma patients who exhibit the greatest clinical benefit from rapamycin-like drugs [170].

Two factors might, however, limit the effectiveness of rapamycin-like drugs in the treatment of renal cell carcinoma. First, the TORC1 complex feedback inhibits signaling by certain receptor tyrosine kinases [184, 185, 185a, 186–188]. As a result, treatment of tumor cells with rapamycin-like drugs can cause a paradoxical increase in receptor kinase activity leading to activation of TORC2, which is relatively rapamycin resistant, PI3K, and AKT, all of which might promote tumor growth [184, 185, 185a, 186–188]. Second, inhibition of TORC1 appears to preferentially inhibit HIF1α, which as argued above appears to act a renal cell carcinoma suppressor, rather than HIF2α [189]. In contrast, inhibition of TORC2 preferentially affects HIF2α [189]. Second-generation, ATP-like, mTOR inhibitors can inhibit both TORC1 and TORC2 and hence might be more active than rapamycin-like drugs in the treatment of clear cell renal carcinoma [190, 191]. Emerging preclinical data support such a view [192].

3.3.5 Treatment of Renal Cell Carcinoma: Angiogenesis Inhibitors

3.3.5.1 VEGF

Renal cell carcinoma is one of the most angiogenic solid tumors. Indeed, renal angiography was once an important tool to diagnose this neoplasm. Renal cell carcinoma hypervascularity reflects the overproduction of HIF-dependent angiogenic factors such as VEGF. Notably, the remarkable upregulation of VEGF observed upon pVHL loss, and consequent increase in new blood vessel production, probably diminishes the selection pressure to upregulate additional angiogenic factors in this setting. In contrast, a host of angiogenic factors in addition to, or instead of, VEGF likely contributes to neoangiogenesis associated with other solid tumor types.

In keeping with this view, a variety of drugs that inhibit VEGF, such as bevacizumab, or its receptor KDR, such as sorafenib, sunitinib, axitinib, and pazopanib, have now demonstrated significant activity in the treatment of renal cell carcinoma and were approved by the FDA [193–197]. These agents induce significant disease stabilization and, in some cases, frank regressions. Newer VEGF inhibitors that are more potent, more specific, or both are in various stages of development. It is anticipated that greater potency will translate into greater clinical efficacy although there might be limits regarding the degree to which VEGF signaling can be safely interrupted in man. Microangiopathic hemolytic anemia was observed in patients in which two VEGF inhibitors were combined [198, 199], and both preclinical and clinical data suggest that chronic VEGF inhibition could lead to cardiomyopathic changes [200, 201]. Developing VEGF inhibitors that exhibit greater specificity is important because some of the existing agents are difficult to combine with other agents, presumably because of their off-target effects. The history of curative cancer therapy suggests that the eventual cure of renal cell carcinoma will require

a combination of agents that have novel mechanisms of action and that are non-cross resistant. A VEGF inhibitor will probably be cornerstone of such a combination.

In the simplest view, pVHL status would serve as a predictive biomarker, with VEGF inhibitors being more active in pVHL-defective renal cell carcinomas than in pVHL-proficient tumors. Although some studies support this contention, others do not [202–205]. This lack of consistency might be due, at least partly, to technical differences related to how pVHL status was determined and how therapeutic response was measured. It appears that the vast majority of clear cell renal carcinomas (especially those that do not exhibit mixed histological patterns with areas of non-clear cell features) have transcriptional signatures indicative of pVHL inactivation and HIF activation, including some without demonstrable *VHL* mutations or hypermethylation [6]. Studies with newer sequencing platforms suggest that some of these tumors do, indeed, have *VHL* mutations that would be missed using conventional DNA sequencing approaches [4, 7]. Suffice it to say that *VHL* status is not currently a sufficient robust predictive biomarker to be used in clinical decision-making.

3.3.5.2 PDGF

Platelet-derived growth factor B (hereafter called PDGF) is another well-studied HIF target [206, 207]. PDGF supports the expansion of pericytes that surround new blood vessels and provide survival signals to the associated endothelial cells. In preclinical models, newly sprouting blood vessels that lack pericyte coverage are more sensitive to VEGF blockade than are more mature vessels that are associated with pericytes [208–210]. This might explain why the objective tumor response (regression) rate in renal cell carcinoma is higher with small-molecule KDR inhibitors, many of which inhibit PDGFR, than with bevacizumab, which solely inhibits VEGF. On the other hand, it should be borne in mind that PDGFR inhibitors such as imatinib mesylate have not yet demonstrated utility as single agents in renal cell carcinoma and have not been shown to enhance the activity of bevacizumab [211–213]. Moreover, many of the existing KDR inhibitors might have off-target effects other than PDGFR inhibition that fortuitously contribute to their antitumor activity.

3.3.5.3 IL-8

VEGF inhibitors, although highly active in renal cell carcinoma, are not curative as single agents, and renal cell carcinoma patients treated with these agents will eventually experience disease progression. The mechanisms underlying de novo or acquired resistance to VEGF inhibitors are poorly understood at the molecular level. One study suggested that upregulation of the angiogenic cytokine IL-8, which cooperates with VEGF in some settings [214], contributes to resistance to VEGF inhibitors [215] and IL-8 polymorphisms and circulating IL-8 levels have been linked to clinical outcomes in patients treated with VEGF inhibitors [216, 217]. Interestingly, IL-8 is regulated by HIF and NFκB, both of which are controlled by pVHL [214, 218–222] (Fig. 3.3). These considerations warrant exploration of inhibitors of IL-8, or its receptors CXCR1 and CXCR2, in renal cell carcinoma.

3.3.5.4 TIE2

The receptor tyrosine kinase TIE2 plays an important role in angiogenesis [223]. Activation of TIE2 by ligands such as angiopoietin 1 stabilizes blood vessels, while antagonists such as angiopoietin 2 destabilize blood vessels, rendering them permissive for sprouting and new blood vessel formation but also hyperdependent on VEGF as a survival factor. Although there have been conflicting reports on the regulation of angiopoietins by pVHL [224, 225], knowledge of TIE2 biology suggests that dual inhibition of VEGF and TIE2 might block angiogenesis more effectively than would VEGF blockade alone. Circulating levels of a soluble form of TIE2 have also been touted as a means of monitoring antiangiogenic therapy in this patient population [226].

Unfortunately, the TIE2 antagonist AMG386 in combination with the VEGFR inhibitor sorafenib was not more active than sorafenib alone [227].

3.3.5.5 CXCR4 and SDF

Both CXCR4 and its ligand, CXCL12/SDF, are HIF targets and upregulated in pVHL-defective tumors [228, 229]. In some mouse models, blocking CXCR4 inhibits the recruitment of circulating bone marrow-derived cells that can contribute to new blood vessel formation and can enhance the antiangiogenic activity of VEGF inhibitors [230]. CXCR4 might also play cell autonomous roles in renal cell carcinoma invasion and metastasis. In this regard, neutralizing antibodies to CXCL12 were shown to decrease metastasis, without affecting angiogenesis, in an orthotopic renal tumor model in mice [231]. Conversely, upregulation of CXCR4 on an epigenetic basis was associated with increased renal cell carcinoma metastasis [232].

3.3.6 Treatment of Renal Cell Carcinoma: Tumor Cell Receptor Tyrosine Kinases

3.3.6.1 EGFR

Renal cell carcinomas frequently overexpress EGFR and its ligand TGFα [233–236]. TGFα is a transcriptional HIF target, while HIF has been reported to increase the rate of EGFR translation [97, 237, 238]. In addition, pVHL loss might decrease the rate of EGFR internalization and recycling [129]. In preclinical models, inhibiting EGFR decreases tumor growth in vivo [239, 240].

Despite these observations, EGFR inhibitors have been very disappointing in the treatment of renal cell carcinoma, both alone and in combination with VEGF inhibitors [241, 242]. Why have EGFR inhibitors failed thus far in the clinic? One possibility, in addition to a possible failure to achieve adequate EGFR inhibition in vivo, stems from recent work showing that c-MET, which is frequently active in renal cell carcinoma (see below), can confer resistance to EGFR blockade [243–245]. Preclinical xenograft studies performed in mice frequently underestimate the importance of c-MET because mouse HGF, the ligand for c-MET, does not activate human c-MET (present on implanted human tumor cells) [246, 247].

3.3.6.2 c-MET

pVHL-defective tumor cells exhibit increased c-MET activity and are hypersensitive to HGF [248–250]. Precisely how pVHL regulates c-MET is somewhat controversial, with some report suggesting c-MET is a HIF target [250–252] and others focusing on the effects of pVHL on signaling downstream of c-MET [248, 249]. Interestingly, activating germline *MET* mutations are linked to the development of papillary renal cell carcinoma [253]. HGF and c-MET play an important role in both tumorigenesis and angiogenesis. pVHL-defective tumor cells are hypersensitive to c-MET loss [254], and inhibition of c-MET might, for the reasons outlined above, augment the activity of EGFR inhibitors. Cabozantinib (XL184), which inhibits both VEGFR and c-MET, demonstrated clinical activity in heavily pretreated renal cell carcinoma patients who had failed prior VEGF inhibitor therapy in a phase 1 study [255]. To what extent these responses were due specifically to c-Met inhibition remains to be determined.

3.3.6.3 IGFR

HIF upregulates IGF-1 and IGF-2 as well as IGFB-2 and IGFB-3 [256, 257]. pVHL, in a HIF-independent manner, downregulates IGFR levels by inhibiting SP1 and the RNA-binding protein HuR [134] and IGFR-dependent signaling through PKCδ [123, 124]. Inhibition of IGFR sensitizes renal cell carcinoma cells to cytotoxic drugs as well as to rapamycin-like drugs [258]. This latter observation might relate to the role of rapamycin in feedback inhibition of receptor tyrosine kinase signaling, as described above. In addition, downregulation of IGFR-1 with shRNA technology decreases VHL−/− renal carcinoma growth in nude mouse xenograft assays [259].

3.3.6.4 ROR2

ROR2 (RTK-like orphan receptor 2) was identified in an unbiased screen for receptor tyrosine kinases that are upregulated and activated by

pVHL loss in renal carcinoma cells [260, 261]. The biological functions of ROR2 are incompletely understood although it has been linked to tumor cell invasiveness through the upregulation of matrix metalloproteinases and may act as a receptor for Wnt ligands. Inhibition of ROR2 in renal carcinoma cells with short hairpin RNAs suppresses tumor growth in orthotopic tumor models [261].

3.3.7 Other Targets

3.3.7.1 Cdk4/6

Deregulation of HIF2α in renal cell carcinoma cells drives the overproduction of the cyclin D1 oncoprotein that, together with the cdk4 or cdk6 kinase, promotes cell-cycle progression [64, 87, 262, 263]. In contrast, hypoxia and HIF activation lowers cyclin D1 levels in most other cell types [87]. Some renal cell carcinomas have also sustained deletions of the INK4A tumor suppressor protein [6, 138, 140], which acts as an inhibitor of cdk4 and cdk6, and pVHL-defective tumor cells appear to be hypersensitive to loss of cdk6 in vitro [254]. Moreover, cdk6 is located on a large region of chromosome 7 that is amplified in a subset of renal cell carcinomas [6]. Downregulation of cyclin D1 with shRNA technology is sufficient to inhibit tumor formation by *VHL−/−* renal carcinoma cells in mouse models [259]. Although a relatively promiscuous cdk inhibitor was relatively ineffective in the treatment of kidney cancer at maximally tolerated doses, newer, more selective cdk inhibitors targeted against cdk4 and cdk6 might now be explored for this indication [264, 265].

3.3.7.2 NFκB

pVHL suppresses NFκB via HIF-dependent and HIF-independent pathways [117–120, 266]. With respect to the latter, pVHL, bound to casein kinase 2, promotes the inhibitory phosphorylation of the NFκB agonist Card9 [120]. NFκB activity is increased in human renal cell carcinoma and might contribute to both tumor development and therapeutic resistance [267, 268]. HIF and NFκB coregulate targets such as cyclin D1 and VEGF, and preclinical studies suggest that inhibiting NFκB activity, such as might be achieved with inhibitors of IKK, would have salutary effects in the treatment of kidney cancer [269].

3.3.7.3 IL6

Renal cell carcinomas frequently overexpress interleukin 6, which is suspected of acting as an autocrine growth factor in this disease [270–272]. Binding of IL-6 to its receptor activates the JAK-STAT pathway that, in turn, can stimulate renal carcinoma cell proliferation [273]. IL-6 was shown to be pVHL responsive in one study [262] and has been implicated as both a prognostic biomarker in clear cell renal carcinoma and as a predictive biomarker for clear cell renal carcinoma patients being treated with the VEGFR inhibitor pazopanib [217]. A neutralizing antibody against IL-6 stabilized disease in approximately 50 % of patients with metastatic renal cancer in a phase 2 study [274].

3.3.8 Carbonic Anhydrase and Lactate Dehydrogenase

HIF1α upregulates a number of genes that promote glycolysis and lactate acid production. This potentially places a burden on pVHL-defective tumor cells to maintain pH homeostasis. Preclinical studies suggest that inhibition of lactate dehydrogenase A or carbonic anhydrase IX, both of which are HIF targets, would be a viable therapeutic strategy for treating pVHL-defective renal cell carcinomas [275–278].

3.3.9 Histone Methylases and Demethylases

Resequencing of renal cell carcinoma genomes has identified mutations affecting enzymes that regulate histone methylation, as described above. In addition, HIF transcriptionally activates a number of histone demethylases including JMJD1A, JMJD2B, and JARID1B [164–169]. In one study, inhibition of JMJD1A with a short hairpin RNA inhibited renal carcinoma growth [168]. Histone methylases and demethylases can, in principle, be inhibited with drug-like small

molecules, and the identification of these enzymes as mutational targets in renal cell carcinoma and other neoplasms is motivating a deeper understanding of their biological functions as well as nascent drug discovery efforts.

3.3.9.1 CTLA4 and PD1

It has been appreciated for decades that renal cell carcinoma has a highly variable natural history and that some patients can experience spontaneous regressions. Although the mechanisms underlying such spontaneous regressions are unknown, a role for the immune system has been suspected. Moreover, immune modulators have been used in the treatment of this disease for many years, including high-dose interleukin 2 [279]. High-dose interleukin 2 can induce durable remissions in patients with metastatic kidney cancer. Unfortunately, this therapy is sufficiently toxic that it should only be given at specialized care centers, and it is impossible to predict the small subset of patients who will achieve such lasting remissions.

A growing appreciation of the signals that are used by tumor cells to evade immune recognition has led to new cancer immunotherapeutic agents, including antibodies directed against CTLA4 and PD1, which are proteins that serve to dampen the immune response. Interestingly, a particular CTLA4 polymorphism was found in one study to be associated with the risk of developing renal cell carcinoma [280].

Anti-CTLA4 has demonstrated activity in the treatment of renal cell carcinoma and is now being explored in combinations [281, 282]. A cautionary note is that acute renal failure was observed when anti-CTLA4 was combined with sunitinib [282]. Early data with anti-PD1 antibodies for the treatment of renal cell carcinoma also appear promising [283–285].

It is not yet known whether pVHL loss influences the recognition of tumor cells by the immune system although VEGF has, itself, been implicated as an immune suppressant [286–288]. Moreover, HIF stimulates the production of adenosine, which can suppress the immune response via the A2A adenosine receptor [289, 290]. Future therapies against renal cell carcinoma will likely involve combinations of agents that directly kill tumor cells with agents that enhance the host immune response.

Conclusions

Renal cell carcinoma is a common cancer that, historically, has been refractory to therapy with standard chemotherapeutic agents and radiation. High-dose interleukin-2 can induce durable remissions in a small subset of patients, but it is impossible to predict which patients will benefit from this toxic and expensive form of therapy. The von Hippel-Lindau tumor suppressor protein, pVHL, is frequently inactivated in clear cell renal carcinoma, which is the most common form of kidney cancer. The knowledge that pVHL inhibits the HIF transcription factor provided a conceptual framework for drugs that inhibit the HIF-responsive gene product VEGF. The clinical activity of mTOR inhibitors might also relate to HIF biology because mTOR regulates HIF synthesis and might also act downstream of VEGF. A number of other HIF-responsive gene products are also known or suspected of playing roles in tumorigenesis and are worthy of exploration as kidney cancer drug targets. Elucidation of the genetic events that cooperate with pVHL loss in clear cell carcinoma will hopefully yield additional targets. In this regard, it is anticipated that the frequent occurrence of mutations affecting chromatin regulatory proteins in clear cell renal carcinoma will create exploitable therapeutic vulnerabilities. Finally, there is a growing appreciation of how, mechanistically, clear cell renal carcinoma subverts immune recognition. The studies, in total, should provide a platform for the design and testing of effective therapeutic combinations for this disease.

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Biomarkers for Renal Cell Carcinoma

4

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Key Points

- The report of the Cancer Genome Atlas on the comprehensive profiling of clear cell RCC set the stage for tumor-based biomarker discovery and validation.
- Prognostic biomarkers can establish risk for disease progression and recurrence, but remain to be prospectively validated.
- Predictive biomarkers must focus on available therapeutic options to maximize relevance to clinical practice and immediacy of implementation.
- Diagnostic and early detection biomarkers have the greatest potential to alter the natural history of RCC, but remain distant from clinical realization.

4.1 Definitions and Categories of Cancer Biomarkers

Cancer biomarkers mainly exist as measurable indicators of a carcinogenic process or of a pharmacologic response to a therapeutic maneuver. They are either produced by tumor cells themselves or by the body in response to cancer. Cancer biomarkers, therefore, may be measured not only in tumor tissues but also in normal tissue or bodily fluids. In this chapter, we will break down the current status of tumor tissue-derived

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_4

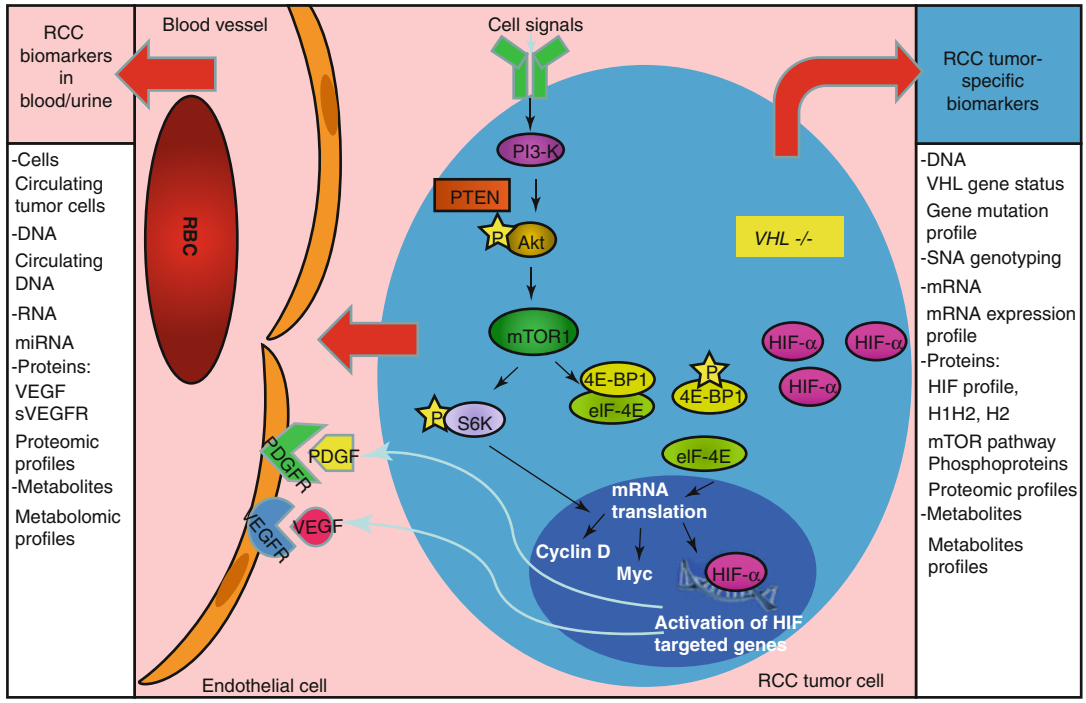

Fig 4.1 Biomarker-relevant biologic pathways in renal cell carcinoma (RCC). In *VHL-/-* tumor cells, the absence of pVHL results in the accumulation of hypoxia-inducible factor alpha (HIF-α). HIF accumulation could also be secondary to the activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway. mTOR phosphorylates and activates pS6K, which leads to increasing translation of downstream target proteins, including cyclin D, Myc, and HIF. Activated mTOR also phosphorylates 4E-BP1, disrupts this complex, and allows eIF-4E to stimulate the mRNA translation as well. Activated HIF translocates into the nucleus and results in the transcription of multiple HIF-target genes, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). These proteins bind to their receptors and cause cell migration, proliferation, and permeability. RCC biomarkers could be derived from cell components of tumor cell itself, including DNA, RNA, protein, and metabolites. The soluble cell components could also migrate from the cell into the blood vessels and be detected in blood and urine of RCC patients

biomarkers, as well as discuss the emergence of blood- or urine-based biomarkers in renal cell carcinoma (RCC).

Biomarkers can be defined according to the following categories:

1. Early detection biomarkers – used to screen patients for cancer
2. Diagnostic biomarkers – used to assess the presence, absence, or type of cancer
3. Prognostic biomarkers – used to evaluate different phenotypes that correlate with clinical behaviors and/or survival outcomes
4. Predictive biomarkers – used to predict individualized response to therapies, especially targeted therapies

A particular biomarker may satisfy more than one category. For example, a circulating tumor marker may aid in early detection and diagnostic clarification and have prognostic or predictive relevance in the management of cancer.

4.2 The Challenge and Opportunity of Cancer Biomarker Development

Thousands of biomarkers are currently in the developmental pipeline as potential markers for cancer detection, diagnosis, prediction of response, and prognosis. Fewer than 25 biomarkers have been approved by the FDA for monitoring response to treatment or for determining recurrence of cancer [1, 2]. Currently, there is no biomarker that is FDA-approved for RCC screening, staging, monitoring, or prognosis.

4.3 The Importance of RCC Biomarker Development

The early detection and diagnosis of RCC remains a challenge to oncologists and presents a significant barrier to reducing mortality due to this cancer. Roughly 30 % of RCC cases present with metastatic disease at the time of initial diagnosis [3]. Although this percentage has declined in recent years due to increased incidental detection of small renal masses, the mortality rate from RCC has remained steadfastly unchanged [4]. This suggests that RCC with lethal potential are not being identified sufficiently early to prevent metastatic spread, and this presents the single most significant opportunity to reduce death due to RCC. Patients with metastatic RCC have a much poorer prognosis compared with patients with early-stage disease, with a 5-year survival rate of 23 % for stage IV disease as compared to a 5-year survival rate of 96 % for stage I presentation [5].

The use of early detection biomarkers remains in development, but interesting tools are on the horizon. New generations of biomarkers that examine novel substrates such as microRNA (miRNA, miR), proteomic, and metabolomic profiles, with the potential to measure hundreds or more elements simultaneously as a biomarker "profile," are being investigated intensely as tools for RCC early detection and diagnosis. The results have been encouraging [6, 7], but await full clinical validation.

Several early detection serum and urinary biomarkers have been reported as a first step toward a clinically relevant RCC detection assay. Noninvasive detection methods are promising given the increased frequency of detection of RCC from incidental findings on imaging. In one recent study, analysis of RCC cases revealed elevated plasma levels of *N*-methyltransferase (NNMT), L-plastin (LCP1), and nonmetastatic cells 1 protein (NM23A) [8]. A three-marker assay was developed with good positive and negative predictive value for RCC, although results of this study remain unvalidated. Examination of urinary samples from newly diagnosed RCC patients and matched controls identified 86 peptides more frequently found in RCC, most of which were fragments of collagen chains. An assay using these peptides was developed and then validated using an independent set of patients, enabling differentiation of RCC from control with excellent discriminative accuracy (AUC of 0.92) [9]. These assays may help indicate the presence of a kidney primary malignancy, although they need to be further validated and studied in a diagnostic capacity.

Metastatic RCC consists of a heterogeneous group of cancers. This creates incredible challenges to prediction of prognosis and response to different therapeutics. Biomarkers have their most immediate potential in RCC to demystify the heterogeneity and classify RCC into meaningful subgroups. Ultimately, having a rational biological signature from which to draw prognostic or predictive information, yet with low cost and minimal specimens from patients, would be invaluable. In the last decade, the emergence of multiple FDA-approved targeted therapies gives promise to patients with advanced RCC; however, it also adds complexity in the effort of tailoring each agent to different individuals in appropriate sequence. Despite increased understanding of the underlying tumor biology of RCC and its variant histologies (which arguably comprise highly distinct disease entities), the current TNM staging and subtyping of RCC give inadequate insight to refine current algorithms for treatment selection, disease monitoring, and management. The identification and utilization of novel biomarkers for prognosis and prediction of response are important approaches for personalized RCC treatment.

4.4 Understanding the VHL Pathway for RCC Biomarker Development

4.4.1 VHL

Before embarking on an inventory of biomarkers for RCC, it is essential to understand the biology and molecular pathways which are known in this disease and from which the majority of biomarkers are derived (Fig. 4.1). A key event in the pathogenesis of clear cell RCC (ccRCC) appears

to be the inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor gene, which is a biallelic event in over 90 % of sporadic ccRCC [10]. The mechanisms that lead to the loss of *VHL* functionality include large-scale and small-scale deletions, missense mutations, early stop codons, truncations, and silencing of the locus by hypermethylation. The *VHL* gene is located on the short arm of chromosome 3. Large deletions of 3p are commonly identified in ccRCC. This causes a loss of heterozygosity in a majority of ccRCCs, leaving cells susceptible to loss of the remaining allele and full inactivation of *VHL* [11]. Overall, the disengagement of *VHL* in this unique tumor type is likely a critical and common event in ccRCC development.

4.4.2 pVHL

The VHL protein (pVHL) performs a critical cellular function in regulating the cellular response to low oxygen. In the presence of sufficient oxygen, pVHL binds to a family of proteins called the hypoxia-inducible factor alpha (HIF-α) subunits, recruiting them to an E3 ubiquitin ligase complex which polyubiquitinates the HIF-α subunits, thus targeting them for proteasome-mediated proteolysis [12]. The loss of pVHL activity, therefore, permits the constitutive stabilization of HIF-α factors, and high-level expression of HIF-α factors has been a widely recognized feature of ccRCC tumor biology. About 90 % of all ccRCC display HIF-α stabilization apparently as a consequence of *VHL* loss or inactivation [13]. Recent evidence has accrued to indicate that pVHL has functions other than regulation of HIF-related pathways, such as regulation of apoptosis, control of cell senescence, and maintenance of the primary cilium [14].

4.4.3 HIF

HIF is a heterodimeric transcription factor complex consisting of an unstable alpha (α) subunit and a stable beta (β) subunit. Three HIF-α genes (HIF-1α, HIF-2α, and HIF-3α) have been identified in the human genome [15]. Both HIF-1α and HIF-2α function as classical transcription factors, although they can also cooperate with additional factors to maximize activity [16]. The role for HIF-3α, which does not clearly act as a transcriptional regulator and exists with many splice-variant isoforms, is poorly understood [17].

Despite many similarities, HIF-1α and HIF-2α are not fully redundant in function. The global gene expression changes induced by HIF-1α and HIF-2α show that they produce overlapping yet distinct gene expression profiles in both cells and in mice [18].

HIF plays a critical role in tumorigenesis. Indeed, there are several lines of evidence that implicate HIF-α and in particular HIF-2α as playing an active role in *VHL*-/- renal cell carcinogenesis. First, RCC-associated pVHL mutants are at least partially defective with respect to HIF-2α polyubiquitination [19, 20]. Genetic manipulation of HIF expression in human tumor cell line xenografts has clearly demonstrated a growth advantage for cells expressing HIF-2α but not HIF-1α [12, 21]. Examination of human ccRCC tissues provided the ultimate demonstration of a dependence on HIF-2α stabilization, showing that all *VHL*-defective RCCs either stabilize dually both HIF-1α and HIF-2α or solely HIF-2α [13]. This observation provides an alternative way of classifying pVHL-deficient tumors based on this distinction of HIF expression. The *VHL* genotype and the protein expression of HIF-1α and HIF-2α proteins were analyzed in 160 primary tumors. The tumors were examined by immunohistochemistry (IHC) for HIF-1α and HIF-2α and messenger RNA profiling. *VHL*-deficient tumors that exclusively express HIF-2α (H2) tumors displayed greater c-Myc activity and higher rates of proliferation relative to those of *VHL*-deficient tumors expressing both HIF-1α and HIF-2α (H1H2), regardless of tumor stage. H2 tumors also demonstrated increased expression of genes involved in DNA repair, decreased levels of endogenous DNA damage, and fewer genomic copy-number changes. Moreover, those *VHL-deficient* H1H2 tumors and *VHL* wild-type tumors displayed increased activation of AKT/mTOR and ERK/MAPK1 growth factor

signaling pathways and increased expression of glycolytic genes. Thus, there may be two biologically distinct types of *VHL*-deficient ccRCC: those that produce HIF-1α and those that do not. The relevance of this distinction as a biomarker remains to be demonstrated, although consistent with expectations, H2 tumors were of a higher T stage than their H1H2 counterparts.

4.4.4 HIF-Responsive Genes

HIF is a potent transcriptional activator of the cellular hypoxia response and more than 100 direct HIF-responsive genes have been described, with a number of these genes active in carcinogenesis [22]. Although some of these genes and their products are being studied in RCC, two deserve special attention: vascular endothelial growth factor (*VEGF*) and carbonic anhydrase IX (*CAIX*, *CA9*).

4.4.4.1 VEGF

VHL-/- ccRCCs are notoriously angiogenic and overproduce a variety of proangiogenic molecules including the HIF-responsive VEGF. VEGF stimulates endothelial cell proliferation, migration, maturation, and survival and is among the most potent endothelial mitogens. Furthermore, the VEGF receptor, kinase insert domain-containing receptor (KDR), may be present on renal carcinoma cells, suggesting the possibility of an autocrine feedback loop, although receptor activity on tumor cells remains to be demonstrated [23, 24].

VEGF and VEGF receptors (VEGFR) have been thrust into the spotlight as a result of substantial activity of targeted therapies, which engage these factors. Bevacizumab is an antibody that binds circulating VEGF protein and has activity in metastatic RCC [25]. In addition, potent tyrosine kinase inhibitors, such as sunitinib, sorafenib, and pazopanib, target the intracellular signaling pathways of multiple members of the VEGF receptor family of proteins. Multiple phase III trials have demonstrated substantial clinical benefit from blocking VEGFRs with sunitinib, sorafenib, and pazopanib [26, 27, 28]. Below, we will discuss the potential utility of biomarkers of VEGF activity in the context of therapeutics that directly target this signaling pathway, either via tumor cells directly or via supporting cells of the endothelium.

4.4.4.2 CAIX

CAIX is a transmembrane protein that may play a role in the regulation of cell proliferation, oncogenesis, and tumor progression. CAIX is a HIF-responsive, hypoxia-induced protein that accumulates in *VHL*-defective RCCs [29]. A study of CAIX expression in 317 primary and 42 metastatic renal neoplasms showed correlation between CAIX expression with ccRCC histology as well as histologic grade, suggesting that this HIF-dependent protein may provide an effective surrogate for HIF stabilization with the potential to independently serve as a biomarker [30].

4.4.5 AKT/mTOR/HIF Pathway

A better understanding of the molecular biology underlying RCC will lead to the development of biomarkers reflecting aberrant signal transduction pathways within these tumors. Mammalian target of rapamycin (mTOR) is a kinase that activates substrates critical for protein synthesis. It directly phosphorylates the ribosomal subunit S6 kinase (S6K) as well as eukaryotic initiation factor 4E (eIF-4E), which is released from its inhibitory binding partner 4E-BP1 upon its phosphorylation by mTOR. Loss of function mutations of the *PTEN* tumor suppressor gene result in increased mTOR activity via AKT-dependent inactivation of the tuberous sclerosis complex (TSC1 and TSC2), and key members of this pathway have been identified to have non-overlapping mutations in a substantial percentage of tumors [31]. Inhibitors of mTOR decrease global translation of proteins including HIF, cyclin D1, and Myc [32]. There are now two FDA-approved mTOR inhibitors used in the clinic for advanced RCC: temsirolimus [33] and everolimus [34], which have led to both improved progression free survival (PFS) and overall survival. The detection of the effector molecules

(phospho S6, phospho 4EBP1, and phospho AKT) has been linked with response to VEGF-targeted therapy [35] and is both prognostic for overall survival and predictive of response to mTOR therapy [36–38].

4.4.6 Other 3p Genes Involved in RCC

In addition to *VHL*, several other genes located on chromosome 3p have been recently shown via massively parallel sequencing to be commonly mutated in RCC. These genes are important in histone modification and chromatin remodeling. The most commonly mutated gene is *PBRM1* (*polybromo 1*) [39]. Histone methyltransferase *SETD2* (SET domain-containing protein) and the histone deubiquitinase *BAP1* (*BRCA1 associated protein-1*) have also recently been described, in addition to several other less commonly mutated genes [40, 41]. There are both positive and negative genetic interactions among these genes, with *PBRM1* mutations and *SETD2* mutations commonly occurring in the same tumor and *PBRM1* and *BAP1* rarely occurring together [42, 43]. These genes, similar to VHL, are tumor suppressor genes in which one allele is typically inactivated by mutation or hypermethylation and the second is inactivated through a large deletion in chromosome 3p, resulting in loss of heterozygosity [44].

BAP1 is a nuclear deubiquitinase and tumor suppressor gene mutated in about 9–15 % of ccRCCs [41, 45–48] (and commonly in other cancers, most notably metastatic uveal melanoma) [49]. It causes expression of a specific gene expression signature and is associated with increased mTOR activation. BAP1 mediates deubiquitination of histone H2A and binds to host cell factor-1 (HCF-1), a component of the chromatin-remodeling complex, and binding is required for suppression of cell proliferation. Therefore, loss or mutation of *BAP1* is thought to result in loss of tumor suppression [41, 45, 50–52]. A missense variant of *BAP1* has also been found as a germline mutation in familial RCC, although it rarely occurs [53].

SETD2 is a two-hit tumor suppressor gene located in the region of chromosome 3p that is deleted in a majority of ccRCCs. It is present in about 7–16 % of ccRCCs [45, 46]. SETD2 functions as a histone modifier and methyltransferase and is responsible for trimethylation of lysine 36 of histone H3, causing decreased H3K36 levels in some tested ccRCC cell lines [54] and thereby possibly influencing gene expression and transcription activation.

PBRM1 encodes a chromatin/nucleosome remodeling complex protein BAF180 and is mutated in 30–45 % of RCC [39, 46]. It is thought to work as a tumor suppressor gene through regulation of DNA accessibility and gene expression and therefore regulate cell proliferation, although the full mechanism of tumorigenesis due to loss of *PBRM1* is not fully understood [42]. Nearly all *PBRM1*-mutated tumors exhibit a hypoxia signature, suggesting a loss of VHL even though not all cases are associated with a detectable *VHL* mutation [39]. Overall, the recently identified mutations of *BAP1*, *PBRM1*, and *SETD2* represent novel genetic contributors to the pathogenesis of ccRCC, a finding that may reveal important prognostic classification groups and potentially inform therapeutic decisions in the future.

4.5 The Development of RCC Biomarkers in Clinical Decision-Making

While biomarkers for early detection and diagnosis remain at an early stage of development, more advances have been made for prognostic and predictive biomarkers of RCC. Here we will focus our discussion on these markers.

4.5.1 Prognostic Biomarkers

Prognostic biomarkers have been studied in parallel with advances in the tumorigenesis of this cancer. A summary of the potential molecular prognostic biomarkers that have been investigated for RCC is provided (Table 4.1). We will focus the following discussion on the broad spectrum of prognostic biomarkers.

Table 4.1 Potential individual molecular prognostic biomarkers for renal cell carcinoma (RCC)

Biomarker	Type	Source	No. of patients	Reference	Results
Circulating tumor cells (CTCs)	Cells	Blood	154	Bluemke et al. [124]	Detection of CTCs was correlated with poor overall survival (RR 2.3; $p=0.048$)
miRNA-106b	RNA	Tumor	38	Slaby et al. [125]	miR-106b is a potential predictive marker of early metastasis after nephrectomy in RCC patients ($p=0.032$)
miR-23b/27b	RNA	Tumor		Ishihara et al. [126]	Lower expression of miR-23b/27b was associated with shorter OS ($p=0.018$ and $p=0.025$, respectively)
Serum amyloid A protein (SAA)	Protein	Blood	119	Wood et al. [127]	Total SAA protein was of independent prognostic significance ($p=0.017$)
Angiotensin receptor type 2 (AR2)	Protein	Tumor	84	Dolley-Hitze et al. [128]	AR2 was overexpressed in the most aggressive forms of RCC and correlates with PFS ($p=0.006$) and cancer stage ($p<0.001$)
CAIX	Protein	Tumor	357	Klatte et al. [83]	CAIX expression was a strong independent prognostic factor for patients with metastatic cc RCC ($p<0.05$)
C-reactive protein	Protein	Blood	282	Jagdev et al. [129]	C-reactive protein was highly significant for cancer-specific survival ($P<0.0001$) and OS ($p<0.002$)
CXCR4, CXCR7	Protein	Tumor	223	D'Alterio et al. [130]	High CXCR4 expression ($p=0.0061$), high CXCR7 ($p=0.0194$) expression, and the concomitant high expression of CXCR4 and CXCR7 ($p=0.0235$) are independent prognostic factors
Cathepsin D	Protein	Urine	239	Vasudev et al. [131]	Cathepsin D showed evidence of independent prognostic value for OS ($p=0.056$)
E-selectin	Protein	Tumor	559	Tran et al. [107]	Higher baseline E-selectin levels associated with better PFS ($p=0.002$)
EZH2	Protein	Tumor	520	Wagener et al. [132]	High nuclear EZH2 expression was an independent predictor of poor cancer-specific survival (HR 2.72, $p=0.025$)
Global histone acetylation	Protein	Tumor	193	Mosashvilli et al. [133]	Global histone modification level was a universal cancer prognosis marker ($p<0.05$)
HGF	Protein	Blood	559	Tran et al. [107]	High baseline level of HGF was associated with worse PFS ($p=0.013$)
HIF-1α	Protein	Tumor	357	Klatte et al. [83]	Patients with high HIF-1α expression (>35 %) had significantly worse survival than patients with low expression (< or =35 %); median survival, 13.5 vs. 24.4 months, respectively ($p=0.005$)

(continued)

Table 4.1 (continued)

Biomarker	Type	Source	No. of patients	Reference	Results
HuR	Protein	Tumor	152	Ronkainen et al. [134]	HuR expression was associated with reduced RCC-specific survival (HR 2.18; $p=0.015$)
IL-6, IL-8	Protein	Blood	559	Tran et al. [107]	High baseline levels of IL-6 and IL-8 were associated with worse PFS ($p=0.021$ and 0.013, respectively)
IMP3	Protein	Tumor	716	Hoffman et al. [135]	IMP3 expression was associated with a 42 % increase in death from RCC ($p=0.024$)
MMP-9	Protein	Tumor	120	Kawata et al. [136]	MMP-9 was associated with high nuclear grade and was an independent prognostic factor ($p=0.003$)
Osteopontin	Protein	Blood	559	Tran et al. [107]	High baseline levels of osteopontin were associated with worse PFS ($p=0.041$)
p-AKT	Protein	Tumor	40	Jonasch et al. [35]	Higher levels of p-AKT were associated with increased OS (HR 1.15, 95 % CI 1.02–1.29)
PI3K	Protein	Tumor	176	Merseburger et al. [137]	Increased PI3K expression was associated with lower survival ($p=0.030$)
p-mTOR	Protein	Tumor	132	Abou Youssif et al. [138]	Cytoplasmic p-mTOR showed independent prognostic significance ($p=0.029$) and fidelity between primary RCCs and their matched metastases ($p=0.004$)
PAI-1	Protein	Tumor	167	Zubac et al. [139]	PAI-1 was a significant prognostic factor of cancer-specific survival ($p<0.001$)
S100A4	Protein	Tumor	32	Bandiera et al. [140]	Five-year survival was lower in patients with high S100A4 expression than weak expression (41 % vs. 78 %; $p<0.05$)
TIMP-3	Protein	Blood	903	Pena et al. [102]	TIMP-3 was the only biomarker prognostic for overall survival in TARGET trial ($p=0.002$)
TS-1	Protein	Tumor	172	Zubac et al. [107, 141]	Thrombospondin-1 expression was associated with high nuclear grade, advanced stage ($p<0.001$), and tumor progression ($p=0.006$)
VEGF-A	Protein	Blood	559	Tran et al. [107]	High baseline VEGF levels were associated with worse OS (p-0.004)

CAIX indicates carbonic anhydrase IX, *CXCR* chemokine receptor, *EZH2* histone-lysine N-methyltransferase, *HGF* hepatocyte growth factor, *HIF* hypoxia-inducible factor, *HuR* the ubiquitous RNA binding protein, *IL* interleukin, *IMP3* U3 small nuclear ribonucleoprotein protein, *MMP9* matrix metallopeptidase 9, *p-AKT* phosphorylated-AKT, *PI3K* phosphatidylinositol 3-kinases, *p-mTOR* phosphorylated mTOR, *PAI-1* plasminogen activator inhibitor-1, *TIMP-3* metalloproteinase inhibitor 3, *TS-1* thrombospondin-1, *VEGF* vascular endothelial growth factor

4.5.1.1 Clinical Biomarkers

Historically, multiple clinical algorithms were used to estimate prognosis, including the UCLA Integrated Staging System (UISS) to predict risk for disease recurrence or disease-associated death [55] and the Memorial Sloan Kettering Cancer Center (MSKCC) risk criteria for estimating survival for patients with metastatic disease [56]. The UISS incorporates the TNM staging systems, performance status, and the Fuhrman grade of the tumor and is heavily weighted based on tumor stage. While valuable, this staging system does little to risk stratify those patients with non-metastatic but sizeable primary tumors. For patients with metastatic disease, which remains incurable with current therapeutic options, the MSKCC algorithm is a valuable clinical tool to establish prognostic intervals for a disease that can range from indolent to rapidly lethal. This system also takes into account the Karnofsky performance status (which can be highly subjective and variable), time from diagnosis to treatment, and laboratory values of hemoglobin, lactate dehydrogenase, and corrected serum calcium. With the widespread clinical use of targeted therapies in RCC, it is necessary for those criteria, which were validated in the era of cytokine therapies, to recruit new biomarkers to match deregulated pathways with effective inhibitors.

In a recent revision of the model, Motzer et al. developed a nomogram that includes both statistically significant and insignificant factors as biomarkers to create a non-biased prognostic model for patients receiving sunitinib [56]. The additional factors included were the number of metastatic sites ($p<0.01$), the presence of hepatic metastases ($p<0.1$), thrombocytosis ($p<0.01$), prior nephrectomy ($p=0.37$), the presence of lung metastases ($p=0.74$), and serum alkaline phosphatase levels ($p=0.82$) [56].

4.5.1.2 Histological Biomarkers

Tumor stage is widely considered by many clinicians as the most important prognostic factor. Historically, effort has focused on identifying critical features in addition to tumor size, such as extracapsular extension, renal vein invasion, inferior venous cava invasion, lymph node involvement, and presence or absence of adrenal gland metastases. It is only recently that the histologic subtyping of RCC into clear cell, papillary, and chromophobe variants gained its long-deserved attention. Aggregation of data has shown that each tumor subtype is associated with different pathophysiology and clinical behavior. In the largest and most comprehensive retrospective review to date, a group of 3,062 cases was identified between 1970 and 2003, among them 2,466 patients (80.5 %) with clear cell, 438 (14.3 %) with papillary, and 158 (5.2 %) with chromophobe RCC. A significant difference in metastasis-free and cancer-specific survival existed between patients with ccRCC and the two other dominant subtypes. Even after multivariate adjustment, the ccRCC subtype remained a significant predictor of metastasis and cancer-specific death [57].

In an effort to estimate prognosis within the ccRCC group, the Fuhrman grading system has been used to further categorize tumors according to tumor cell morphology and correlates tumor grade to mortality [58]. Other histologic features, including the presence of alveolar features, lymphovascular invasion [59], and sarcomatoid dedifferentiation [60] play pivotal roles in prognosis as well, although the degree to which each of these affect prognosis is uncertain.

4.5.1.3 Genetic Biomarkers

Traditional cytogenetic karyotyping studies have altered the approach used in classifying RCC subtypes. Characteristic karyotypes have been consistently associated with each of the most common subtypes of RCC (clear cell, papillary, and chromophobe) [61–63]. In ccRCC, the most frequently observed cytogenetic abnormalities were loss of 3p (60 %), gain of 5q (33 %), loss of 14q (28 %), trisomy 7 (26 %), loss of 8p (20 %), loss of 6q (17 %), loss of 9p (16 %), loss of 4p (13 %), and loss of chromosome Y in men (55 %) [64]. It is interesting that tumors with loss of 3p typically presented at lower TNM stages. Loss of 4p, 9p, and 14q were all associated with higher TNM stages, higher grade, and greater tumor size. A deletion of 3p was associated with better prognosis, while loss of 4p, 9p, and 14q were

each associated with worse prognosis [64]. With regard to the less common RCC variants, in papillary RCC, trisomies of chromosomes 7 and 17 were found to be specific genetic alterations irrespective of their size, grade, and cellular differentiation [65]. Another study indicated trisomy 16 and chromosome Y were specifically involved in papillary RCC [66]. The rarest subtype of the three, chromophobe RCC, predominantly showed loss of whole chromosomes, such as loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 [67]. A recent evaluation of the somatic mutation spectrum of chromophobe RCC showed these tumors have commonly mutated *TP53* and *PTEN* genes, although less than half of all tumors had one of these mutations [68]. Further analysis revealed frequent *TERT* promoter genomic rearrangements in chromophobe RCC, as well as alterations in mitochondrial DNA including increased mitochondrial genome copy numbers and electron transport gene complex 1 mutations [68].

Karyotyping provides a piece of the genetic puzzle of RCC tumorigenesis by elucidating some chromosomal changes. However, in order to complete the puzzle and identify the stepwise progression of RCC carcinogenesis, we have to rely on genomic or exomic sequencing, array comparative genomic hybridization (a-CGH), or SNP analysis.

Recent advances in sequencing technology have made large-scale genomic sequencing rapid and cost-effective. As above, several genes located on chromosome 3p (*PBRM1*, *SETD2* and *BAP1*) have recently been identified as commonly mutated in ccRCC, along with the frequently mutated *VHL* gene. These results indicate that large-scale gene sequencing is no longer limited by cost and can provide substantial genetic information to identify heterogeneity in ccRCC.

The presence of these genetic mutations has been shown to have prognostic and predictive significance. Patients with *BAP1*-mutated tumors have significantly worse median overall survival with a nearly threefold increased hazard ratio for death than those with *PBRM1* mutations [50]. *BAP1* is also an independent marker of poor prognosis in patients with low-risk disease and may be able to help risk stratify this group of patients [51]. Presence of *BAP1* is also associated with metastatic disease at presentation [45]. The combination of *BAP1*- and *PBRM1*-mutated tumors is rare and has been associated with an even worse overall survival than either mutation alone in most studies, although not in one small study [45, 50]. The *BAP1* mutation was originally described via genetic sequencing [41], but immunohistochemical testing has now been validated and also correlates with poor overall survival and adverse clinicopathological tumor features [52]. *SETD2* mutations are associated with worse cancer-specific survival in a cohort of patients from the Cancer Genome Atlas, but not an MSKCC cohort [46]. The presence of *PBRM1* mutation does not seem to be associated with a change in cancer-specific survival [47], although it has been associated with advanced tumor stage in some earlier studies [69]. It therefore has been suggested to play a more prominent role in tumor initiation instead of disease progression [46].

4.5.1.4 Gene Expression Profiles

Multiple studies have used traditional gene profiling using RT-PCR to quantify RNA expression. In 2001, Takahashi et al. studied the expression profile of 29 ccRCC samples and found 51 genes, which could categorize RCC for prognostic purposes [70]. More recently, an analysis of gene expression profiles using machine learning algorithms refined the notion that more than one type of ccRCC was present and used 49 ccRCC samples to define a panel of 120 genes which can accurately define two groups of ccRCC, designated ccA and ccB [71]. This model was refined for application using a NanoString platform using archival renal tumor tissues, demonstrating the feasibility of the approach and showing an advantage of molecular classification using the ClearCode34 biomarker for ccA and ccB integrated with stage and grade over conventional clinical algorithms [72].

Using an RT-PCR platform adapted for fixed tissue analysis, 931 archival formalin-fixed tumor tissues from patients with localized ccRCC were examined across 732 candidate genes [73]. With a median follow-up of 5.6 years, 448 genes were found to be associated with a longer

recurrence-free interval ($p<0.05$). Sixteen genes had a strong association after consideration of clinical pathologic covariates and false discovery adjustments (HR 0.68–0.80). Among the 16 genes, increased expression of angiogenesis-related genes (*EMCN* and *NOS3*) was associated with lower risk of recurrence, as was increased expression of immune-related genes (*CCL5* and *CXCL9*). This profile provides a feature set readily adaptable to validation studies and has additional promise as a potential predictive biomarker as well. Several of the recently discovered 3p genes commonly mutated in ccRCC also have unique gene expression profiles, but they have been thus far indistinguishable from nonmutant tumors using unsupervised hierarchical clustering algorithms and are therefore not ready for clinical use at this time [42].

4.5.1.5 Hybrid Strategies

The current trend is to incorporate multiple complementary approaches for better identification and understanding of cancer-related genes. Cifola et al. performed the first integrated analysis of DNA and RNA profiles of 27 RCC samples [74]. Seventy-one differentially expressed genes (DEGs) were found in aberrant chromosomal regions and 27 upregulated genes in amplified regions. Among them, the transcripts encoding *LOX* and *CXCR4* were found to be upregulated. Both are implicated for cancer metastasis. Such combinations of genomic and transcriptomic profiling may potentially provide us a more powerful tool for prognostic estimation.

Another trend is to combine epigenetic data with gene expression profiling for better understanding of these interactions. In a preliminary study, an 18-gene promoter methylation panel using quantitative methylation-specific PCR (QMSP) for 85 primarily resected RCC was evaluated [75]. Significant differences in methylation among the four subtypes of RCC were found for *CDH1* ($p=0.0007$), *PTGS2* ($p=0.002$), and *RASSF1A* ($p=0.0001$). *CDH1* and *PTGS2* hypermethylation levels were significantly higher in ccRCC compared to non-ccRCC. *RASSF1A* methylation levels were significantly higher in papillary RCC than in normal tissue ($p=0.035$). Further validation of epigenetic data in larger cohorts is needed to explore the true prognostic value.

4.5.1.6 Copy-Number Analysis

Array comparative genomic hybridization (a-CGH) has been used to identify the specific copy number changes associated with RCC. A comprehensive analysis incorporated a-CGH and gene expression profiles from 90 tumors in order to identify new therapeutic targets in ccRCC [76]. There were 14 regions of nonrandom copy-number change, including seven regions of amplification (1q, 2q, 5q, 7q, 8q, 12p, and 20q) and seven regions of deletion (1p, 3p, 4q, 6q, 8p, 9p, and 14q). An analysis aimed at identifying the relevant genes revealed *VHL* as one of three genes in the 3p deletion peak, *CDKN2A* and *CDKN2B* as the only genes in the 9p deletion peak, and *MYC* as the only gene in the 8q amplification peak. An integrated analysis to identify genes in amplification peaks that are consistently overexpressed among amplified samples confirmed *MYC* as a potential target of 8q amplification and identified candidate oncogenes in the other regions.

a-CGH may also improve the diagnostic accuracy for RCC. A recent study examined a-CGH on ex vivo fine-needle aspiration (FNA) biopsies and tumor fragments of 75 RCC patients. The pattern of genomic changes identified by a-CGH was used blindly to classify the renal tumors and the genetic findings were subsequently compared with the histopathologic diagnosis. a-CGH was successful in 82.7 % of FNA biopsies and in 96 % of tumor fragments. The genetic pattern correctly recognized 93.5 % of ccRCC, 61.5 % of chromophobe RCC, 100 % of papillary RCC, and 14.3 % of oncocytoma, with the negative predictive value being above 90 % [77]. As RCC histology is an independent predictor of prognosis, one could postulate that a-CGH will have powerful prognostic value as well.

4.5.1.7 SNP Genotyping

Single nucleotide polymorphism (SNP) genotyping has been used to detect cytokine gene

polymorphisms in RCC patients to determine its prognostic significance. A panel of 21 SNPs within the promoter regions of 13 cytokine genes were analyzed in a single-center study of 80 metastatic RCC patients [78]. IL4 genotype -589T-33T/-589C-33C was identified as an independent prognostic risk factor in metastatic RCC patients with a median overall survival decreased 3.5-fold (3.78 months, $p<0.05$) compared with patients homozygous for IL4 haplotype -589C-33C (13.44 months). An association was also found between three SNPs (−2578C/A, −1154G/A, and −634C/G) in the VEGF gene and survival of 213 RCC patients [79]. A more recent study found an SNP in IL-8 was associated with survival in patients treated with pazopanib, and these results were validated using data from the COMPARZ trial in sunitinib-treated patients [80, 81]. Multiple VEGF SNPs have also been associated with response and survival as well [80, 82]. These studies contribute evidence that SNP genotyping could be used to develop prognosis algorithms in patients with metastatic RCC.

4.5.1.8 VHL and HIF as Prognostic Biomarkers

Based on the extensive discussion of the derangement of this pathway as a result of *VHL* mutation, it is not surprising then that *VHL* loss or HIF stabilization might provide a prognostic resource. Perhaps owing to the high prevalence of *VHL* mutation among ccRCCs, numerous efforts to demonstrate that *VHL* mutation is a prognostic indicator have been unfruitful. Klatte and colleagues showed preliminary evidence that HIF-1α expression can provide an independent prognostic factor for patients with ccRCC. Patients with high (>35 %) tumor immunostaining of HIF-1α had shorter survival than patients with low (≤35 %) immunostaining of HIF-1α [83]. However, more recent studies have suggested that higher expression of HIF-1α and HIF-2α are associated with improved prognosis [84, 85]. Whether tumor expression of HIF-1α provides substantial prognostic information with respect to the natural history of ccRCC remains to be determined, as does the role of HIF-2α in this setting.

4.5.1.9 Circulating Cells

Levels of circulating endothelial cells and circulating tumor cells have been recently gaining attention as prognostic biomarkers. Several studies have shown that higher levels of circulating endothelial cells or circulating endothelial progenitor cells during the first cycle of VEGF-targeted therapy were associated with improved PFS [86, 87]. However, this technology remains investigational for assessing disease at this time.

4.5.2 Predictive Biomarkers

With the abundance of approved therapies for RCC, oncologists now have the luxury to choose individualized therapy for each patient. Traditional immunotherapy should be retailored to fit selected patients better. Targeted therapies not only have invigorated RCC oncologic practice but also have changed the approaches used to predict response to therapy and to measure clinical outcome. In the next section, we differentiate and discuss biomarkers according to different therapies (Table 4.2).

4.5.2.1 Predictive Biomarkers for Immunotherapy

Despite the advances of targeted therapy, traditional immunotherapy is not obsolete. Immunotherapy offers the possibility of a complete and durable response for a small number of patients with favorable disease factors. However, the toxicities from immunotherapy are significant and the disease factors, which favor immunotherapy, are uncertain. Immunotherapy is therefore often not considered a reasonable option. A reliable biomarker would be ideal to select patients who are likely to have a good response or less toxicity to immunotherapy, as well as to monitor their progress. In addition, the introduction of anti-PD1 (programmed death-1) therapy could also uncover predictive biomarkers for this therapy in the near future.

RCC Subtyping

It is clear that RCC subtyping for clear cell histology is an important predictive biomarker for immunotherapy [88–90]. The Cytokine Working Group performed a retrospective analysis of

Table 4.2 Potential predictive biomarkers of response to targeted therapies for renal cell carcinoma (RCC)

Drug	Biomarker	Reference
Immunotherapy		
IL-2	Clear cell histology	Upton et al. [90]
		McDermott et al. [91]
	CAIX	Bui et al. [142]
	Gene expression profiles	Pantuck et al. [32]
Antiangiogenic therapy		
Sunitinib	Soluble VEGFR	Deprimo et al. [110]
	NGAL, VEGF	Porta et al. [108]
	bFGF	Tsimafeyeu et al. [143]
	HIF-2α	Patel et al. [103]
	TNF-α, MMP-9	Perez-Garcia et al. [144]
	VHL WT	Choueiri et al. [99]
	CXCR4	D'Alterio et al. [112]
Sorafenib	Serum VEGF	Bukowski et al. [106]
		Pena et al. [102]
	TGF-β1 mRNA	Busse et al. [145]
	CAIX	Choueiri et al. [99]
	Osteopontin	Zurita et al. [113]
	VHL loss	Choueiri et al. [99]
Pazopanib	HGF, IL-6, IL-8	Heymach et al. [146]
	IL-6	Tran et al. [107]
Axitinib	*VHL* WT	Choueiri et al. [99]
Bevacizumab	Serum VEGF	Bukowski et al. [147]
	VHL loss	Choueiri et al. [99]
	IL-6, HGF	Nixon et al. [148]
mTOR inhibitors		
Temsirolimus	Non-clear cell histology	Dutcher et al. [114]
	LDH	Armstrong et al. [149]
	p-AKT, pS6K	Cho et al. [92, 122]
Everolimus	LDH	Motzer et al. [150]

IL indicates interleukin, *CAIX* carbonic anhydrase IX, *VEGFR* vascular endothelium growth factor receptor, *NGAL* neutrophil gelatinase-associated lipocalin *bFGF* basic fibroblast growth factor, *HIF* hypoxia-inducible factor, *TNF* tumor necrosis factor, *MMP* matrix metallopeptidase 9, *VHL* von Hippel-Lindau gene, *CXCR* chemokine receptor, *TGF* transforming growth factor, *WT* wild type, *HGF* hepatic growth factor, *LDH* lactate dehydrogenase

tumor tissue from 231 RCC patients treated with interleukin (IL)-2 immunotherapy. The response rate to IL-2 was 21 % in patients with ccRCC, compared with 6 % with non-ccRCC [90]. Similar results were found in the SELECT trial, with zero out of five patients responding [91]. Among the patients with ccRCC, those with >50 % alveolar and no granular or papillary feature had the best response to IL-2 [90].

CAIX

CAIX expression had initially been reported as a predictive biomarker of response to IL-2 [32, 92]. High CAIX expression (>85 % of tumor cells) was observed in 78 % of patients responding to IL-2, compared with only 51 % in nonresponders after examination of 66 RCC patients (27 responders). However, the role of CAIX as a predictive biomarker was further studied in the prospective SELECT trial in combination with histologic features but failed to predict responsiveness to IL-2 [91].

Genetic Studies

Genetic studies as predictive biomarkers have also been explored for immunotherapies. Pantuck

and colleagues reported an expression panel of 73 genes potentially useful to identify complete responders from nonresponders after IL-2 therapy [32]. Interestingly, complete responders to IL-2 possessed unique expression patterns of genes including CAIX, PTEN, and CXCR4. An analysis of a-CGH in ccRCC showed that tumors from complete responders to IL-2 had fewer whole chromosome losses than nonresponders. The loss of chromosome 9p was present in 65 % of nonresponders vs. 0 % of complete responders [93]. Pioneering work using SNP genotyping to predict the response to IFN-α has also been reported [94]. A stepwise logistic regression analysis revealed that the SNPs in signal transducer and activator 3 (STAT3) were significantly associated with better response to IFN-α. All of these findings from exploratory retrospective analyses remain to be validated in prospective studies.

PD1

PD1 (programmed death) is a T-cell immune checkpoint receptor thought to be involved in tumor-mediated immunosuppression. Preliminary data from phase II studies suggest that patients with RCC that expresses PDL1 (the ligand that binds T-cell PD1) on their tumors may benefit from anti-PD1 therapy more than those without, although these results need to be validated in future studies [95].

4.5.2.2 Predictive Biomarkers for VEGF-Targeted Therapy

Clinical Biomarkers

It is intriguing to note that hypertension (HTN), a frequent side effect of VEGF-targeted therapy, has been strongly associated with clinical outcome in the setting of VEGF-directed agents. Rini et al. reported that HTN could be used as a predictive biomarker of efficacy in patients treated with sunitinib [96]. Patients with a maximum systolic blood pressure (SBP) of 140 mmHg or more had a greater improvement in both PFS (12.5 vs. 2.5 months; $p<0.0001$) and OS (30.5 vs. 7.8 months; $p<0.0001$), when compared with patients with lower SBP. Similar results were found in studies of interferon and bevacizumab treatment when patients who developed grade 2 or more HTN had both improved PFS and OS [25, 96–98].

VHL Mutation

VHL gene mutation is a key event of tumorigenesis of ccRCC, a highly vascular neoplasm. Although the incidence of this lesion is >90 %, it has been postulated that *VHL* gene status may serve as a predictive biomarker for ccRCC patients in monitoring of response to VEGF-targeted agents. Recently, Choueiri et al. examined 123 ccRCC patients treated with VEGF-targeted monotherapy with sunitinib, sorafenib, axitinib, or bevacizumab [99]. In multivariate analysis, patients with *VHL* mutational events obtained a significant response rate of 52 % (when missense mutations were excluded) compared to those with wild-type *VHL* who had a response rate of 31 % ($p=0.04$). Interestingly, no responses were noted in patients with wild-type *VHL* receiving sorafenib or bevacizumab. However, *VHL* mutation status did not seem to affect the responses seen in patients treated with potent VEGFR inhibitors sunitinib or axitinib. Other small studies did not provide strong evidence to support the predictive value of *VHL* mutation as a biomarker. In 13 RCC patients treated with axitinib, no correlation was seen between somatic *VHL* mutational status and response [100]. In another study, *VHL* gene status of 78 RCC patients treated with pazopanib was examined, but no association was found between *VHL* gene status and response [101]. *VHL* mutational status did not predict treatment benefit in a large phase III study of sorafenib in advanced RCC, although only a minority of patients had known *VHL* mutation status [102]. Taken together, it remains uncertain whether any correlation exists between *VHL* status and VEGF therapy response, and definitive studies are awaited.

HIF Levels

Patel and colleagues used Western blot to measure HIF expression level in 43 ccRCC specimens prior to sunitinib treatment. Twelve (92 %) of 13 patients with high HIF-2α expression

(>50 % compared to cell line control) responded to sunitinib, whereas only 4 (27 %) of 15 patients with low expression of HIF-2α showed response to sunitinib [103]. A recent abstract reported that both HIF-1α and HIF-2α (H1H2)-positive expressions were correlated with improvement in PFS and OS, as well as response rate to first-line VEGF TKI therapy [84]. This is somewhat contradictory to a previous study by Klatte et al. that showed patients with higher expression levels of HIF-1α had significantly worse overall survival than those with low expression [83]. Studies further establishing the role of classifying tumors according to HIF expression profile have been hindered by technical limitations of antibody nonspecificity, rapid oxidation, and degradation of HIF proteins in improperly handled specimens. In addition, microdeletions in HIF-1α in some cases can lead to nonfunctional protein that retains the domains and features for antigen detection by traditional immunostaining [104].

VEGF/Soluble VEGF Receptor Levels

The value of plasma VEGF levels as a predictive biomarker for antiangiogenesis therapies was addressed in the TARGET trial [105, 106]. High baseline VEGF level was an independent prognostic factor ($p=0.014$) as patients with high baseline VEGF had poorer prognosis. This has been validated in several other trials [107–109]. In another trial, both patients with high VEGF levels and low VEGF levels at baseline benefitted from sorafenib therapy, although those with high VEGF levels had a trend toward more pronounced benefit [102].

A phase 2 trial investigating circulating biomarker changes after sunitinib treatment in cytokine-refractory disease demonstrated significant changes in VEGF, sVEGFR-2, and sVEGFR-3 levels in patients with objective tumor response compared with those with stable disease or disease progression [110, 111]. This finding was similar to findings that lower baseline levels of sVEGFR-3 and VEGF-C were associated with longer PFS and better tumor response in patients receiving sunitinib following disease progression on bevacizumab [109]. Similarly, biomarker studies in a phase 2 trial with pazopanib showed that sVEGFR-2 decrease at day 14 of therapy predicted a better outcome in terms of response and PFS [101].

There has also been some evidence of cross talk between the VEGF pathways and CXCR4 pathway, and one small study has suggested that low CXCR4 expression correlates with improved responsiveness to sunitinib therapy [112].

Cytokines and Angiogenic Factors

Thus far, no single cytokine or angiogenic factor has emerged as reliably predictive of response to VEGF-targeted therapy. However, several studies have explored using clusters of cytokines and angiogenic factors (CAFs) to predict response to therapy. One study found a six-marker baseline signature of factors correlated with improved PFS on sorafenib [113]. However, another study showed no difference in PFS or OS with pazopanib treatment based on CAF signature with similar included factors [107].

4.5.2.3 Predictive Biomarkers for mTOR-Targeted Therapy

RCC Subtyping

RCC subtyping could be an important predictive biomarker for mTOR inhibitors as well. In contrast to immunotherapies, mTOR inhibitors seem more effective in non-ccRCC.

In a subset analysis of a randomized phase 3 trial, median overall survival of patients with non-ccRCC (75 % of whom had the papillary subtype) was 11.6 months in the temsirolimus group vs. 4.3 months in the IFN group [114]. The favorable activity of temsirolimus in non-ccRCC is also different from what was observed with the VEGFR antagonists sorafenib and sunitinib, both of which have demonstrated only limited activity against non-ccRCCs [115]. In the RECORD-3 trial, patients with non-ccRCC had worse PFS than ccRCC when treated with either sunitinib or everolimus as first-line therapy [116]. Patients with non-ccRCC had a longer PFS on first-line sunitinib than everolimus (7.2 vs. 5.1 months), suggesting that perhaps mTOR inhibitors are not more effective than VEGF inhibitors in the non-ccRCC subtypes. A study randomizing patients with non-clear

cell histologies between sunitinib and everolimus showed superiority of sunitinib over everolimus [Ref]. The ongoing phase II ASPEN trial comparing sunitinib and everolimus in non-ccRCC will potentially confirm these findings.

PTEN Loss

The tumor suppressor gene *PTEN* (phosphatase and tensin homologue) encodes a dual specific protein and phospholipid phosphatase that is involved in tumorigenesis and is one of the most commonly lost tumor suppressors in human cancer. It has been reported that *PTEN* loss could be associated with poor prognosis in RCC [117], although interest has focused on *PTEN* deletion as a potential indicator of response to mTOR inhibitor therapy. However, clinical studies have not substantiated either the prognostic role of *PTEN* loss in RCC or any correlation between tumor PTEN expression to either tumor response, OS, or PFS in patients treated with temsirolimus [116–118].

Phospho AKT/Phospho S6K

AKT regulates cell growth and survival mechanisms by phosphorylating a wide spectrum of cellular substrates, including mTOR [119]. Previously, phospho AKT (p-AKT) expression was shown to be correlated with pathologic variables and survival, with higher levels of cytoplasmic p-AKT expression compared with nuclear p-AKT in primary RCC [120]. A recent study found cytoplasmic p-AKT to be significantly correlated to other pathway markers and to nuclear p-AKT in RCC metastases. Unlike primary RCC, p-AKT staining was not prognostic in that cohort of RCC patients [121]. Recent clinical trial data showed that a higher level of p-AKT is associated with both decreased PFS and OS in general in patients with RCC [35].

When mTOR is activated, it phosphorylates two proteins, 4E-BP1 and S6 kinase, to start the cell cycle protein translation process. In primary RCC, phospho S6 kinase (pS6K) expression has been associated with T stage, nuclear grade, incidence of metastasis, and cancer-specific survival [120]. Cho and colleagues investigated *VHL* mutation, p-AKT, and pS6K expression in archival tumor specimens from 20 RCC patients treated with temsirolimus [122]. Although there was no correlation seen between *VHL* mutation and treatment response, protein expression of p-AKT and pS6K, two important proteins indicating activity of the mTOR pathway, was positively associated with response to mTOR-directed treatment. This has been further validated in recent studies with correlation of p-4E-BP1 expression with PFS on mTOR therapy [37]. Another study found that phosphorylation of mTOR and S6RP (the 40S ribosomal protein S6 which increases mRNA transcription in response to mTOR activation) was related to response to mTOR therapy (PFS). However, that study did not show a correlation between expression levels of p-4E-BP1 and efficacy of mTOR therapy [38].

Genetic Biomarkers

A recent case series explored the genetic signatures of several patients who were long-term responders to mTOR inhibitor therapy. Genomic alterations with an activating effect on mTOR signaling were detected in 11 of 14 specimens through alterations in two genes (*TSC1* and *MTOR*) [123].

4.5.2.4 Predictive Biomarkers for Other Targeted Therapies

MET

MET germline mutations have been suggested to play a predictive role in response to new MET inhibitor or multikinase inhibitor therapy in papillary RCC. A recent phase II trial showed up to 50 % partial response rate in papillary RCC with *MET* germline mutations compared to 9 % in those without mutations [167]. These results need to be validated with further studies, but provide one of the most promising rational biomarker/therapy combinations on the horizon.

4.6 Biomarkers on the Horizon

The advent of new technologies and new capacity to bring together these novel methodologies with robust clinical studies heralds a tremendous opportunity for the next generation of biomarkers, reviewed in Table 4.3.

Table 4.3 Potential biomarkers on the horizon for renal cell carcinoma (RCC)

Biomarker	Reference	Mechanism	Potential role in RCC
Proteomic profiles			
Proteomic analysis alone	Xu et al. [151]	High sensitivity and specificity in identifying new proteins or protein amount changes	Early detection, diagnosis
Combined studies	Seliger et al. [7, 152]	Comprehensive analysis of molecular signatures	Early detection, diagnosis
Cytokine and angiogenic factors (CAF)	Zurita et al. [113]	Profile of cytokine and angiogenic factor protein expression levels	Prognostic, potentially predictive for therapy response
Metabolomic profiles			
Tumor specific	Catchpole et al. [153]	Reveals key metabolic features of RCC	Early detection, diagnosis
Body fluids (blood, urine)	Zira et al.; Kim et al. [154, 155]	Easy access, high throughput	Early detection, diagnosis
MicroRNA			
microRNA profile alone	Heinzelmann et al. [156]	miRNA signature may distinguish between metastatic and nonmetastatic ccRCC	Prognosis
Combined RNA studies	Liu et al. [157]	Identify direct mRNA targets of microRNA dysregulated in RCC	Diagnosis
Combined with other studies	Seliger et al. [7]	Comprehensive analysis of molecular signatures	Early detection, diagnosis
DNA			
Circulating cell-free DNA	Feng et al. [158]	Identifies circulating cell-free DNA by PCR, correlates with chance of remission	Response monitoring
Noninvasive imaging biomarkers			
PET imaging			
^{18}F-fluorodeoxyglucose	Minamimoto et al. [159]	Glucose uptake in tumor cells	Staging, response monitoring
^{124}I-cG250	Divgi et al. [160]	cG250 is a monoclonal antibody against CAIX	RCC subtyping, staging
MRI			
Conventional MRI	Spero et al. [161]	Higher sensitivity than renal CT	Staging, subtyping
Modified MRI	Wang et al. [162]	Diffusion-weighted imaging provides images weighted with the local microstructural characteristics of water diffusion	Tumor vascularity assessment and response monitoring
	Hillman et al. [163]	Dynamic contrast-enhanced monitor vascular changes induced by therapeutic agents	
	Pedrosa et al. [164]	Arterial spin labeling uses magnetic fields to label water protons in arterial blood and measures blood flow into tissue	
Magnetic resonance spectroscopy (MRS)	Katz-Brull et al. [165]	Tumor related molecular environment changes cause signal frequency changes	Metabolic portrait of tumors
Ultrasound			
Contrast-enhanced	Lassau et al. [166]	Tumor vascularity	Tumor vascularity assessment and response monitoring

4.7 The Future of RCC Biomarkers Development

Unprecedented progress has been made for RCC biomarker development. However, challenges remain. Most clinical biomarkers need further clinical validations, especially in prospective studies. The bulky panel of potential genetic biomarkers, which we obtained from genomic, proteomic, metabolomic, and microRNA profiling, require further analysis and validation to be useful. Newer biomarkers detectable in serum, urine, and other body fluid need fine-tuning to be isolated from confounding factors. The advancement of therapy for RCC to a new era will undoubtedly involve individualized treatment using biomarkers.

Clinical Vignette

A 66-year-old man underwent a laparoscopic left nephrectomy of a 9-cm renal cell carcinoma (RCC). Three years later, he presents to his oncologist with a 2-month history of nonproductive cough, a 10-lb weight loss, and left hip pain. Further workup reveals multiple pulmonary nodules and a 3×3-cm left sacral lytic lesion. Biopsy of the sacral bony lesion confirmed recurrent RCC. What clinical or biological indicators are needed to determine the most appropriate next step for management of this patient at this time?

This patient has metastatic RCC, and additional information is needed to estimate his prognosis and select the best possible therapy at this time. First, we know that 3 years went by before he developed symptomatic evidence of metastatic disease. To fully assess his prognosis using the Memorial Sloan Kettering risk criteria, we also need to know his performance status and laboratory measures of hemoglobin/hematocrit, corrected serum calcium, and serum lactate dehydrogenase. To refine the risk estimate if the patient is being considered for VEGF receptor-targeted therapy, we also need to know his alkaline phosphatase level and platelet count. This prognostic assessment is invaluable in making plans for treatment and for patients and their families to prepare for the future. The clear cell, papillary, or chromophobe designation becomes essential as therapeutic choices are made between cytokine, VEGF-targeted, and mTOR inhibitor therapies. Other pathologic considerations such as tumor grade, sarcomatoid histology, or alveolar clear cell features also factor into decision-making. To date, none of the molecular markers described above are available as clinical tests to enable earlier detection of this patient's metastatic disease, to further refine his prognosis, or to provide a clear guidepost for therapeutic selection. Patients like the one described above should be encouraged to participate in clinical trials that incorporate biomarker discovery or validation.

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Genetic Heterogeneity of Kidney Cancer

5

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Contents

Key Points

- The observation that renal cell cancer patients often develop mixed responses to therapy has led to the hypothesis that intratumoral heterogeneity may exist within individual patient tumors.
- Advances in molecular phenotyping techniques have led to the identification of significant intratumoral genetic heterogeneity in renal cell cancers of clear cell and variant histologies.
- Phylogenetic trees constructed by inferring ancestral relationships of tumor subclones demonstrate branched rather than linear evolution patterns in individual renal cell cancers. The majority of known driver mutations in renal cell carcinoma map to branching and not to truncal portions of phylogenetic tree constructions.
- Individual renal cell cancers demonstrate evidence of convergent phenotypic evolution by tumor subclones. SETD2 and other tumor suppressor genes have undergone distinct genetic alterations in multiple spatially separated regions within a single tumor converging on loss of function.
- Intratumoral heterogeneity in renal cell cancer may confound clinical

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_5

decision-making on therapeutic strategies, alter drug development strategies, and may require the identification of improved biomarkers to guide clinical practice.

5.1 Background

Clinicians have long suspected that significant heterogeneity may exist within individual tumors and their metastases [1]. Patients with metastatic renal cell cancer (RCC) are known to develop mixed responses to therapy suggesting the presence of tumor subclones and clonal selection [1, 2]. In the past, traditional laboratory techniques were employed to gain insights into the molecular basis of such heterogeneity. For example, chromosomal analysis has shown that a more complex cytogenetic pattern is found in more aggressive and advanced RCC, suggesting that sequential accumulation of chromosome changes may play a role in cancer progression [3]. An evaluation of chromosomal mutations and mitotic segregation patterns in RCC showed that in a subset of tumors, there were abnormally shortened telomere repeat sequences, chromosomal breakage-fusion-bridge events, multipolar configurations, and supernumerary centrosomes [4]. These observations suggested that changes in cell division machinery may be involved in the evolution of complex karyotypes and genetic intratumoral heterogeneity in a subgroup of RCC. Furthermore, Ljundberg et al. employed flow cytometry to evaluate DNA ploidy in 200 consecutive RCC specimens: these investigators reported that there was frequent heterogeneity in these specimens and concluded that "multiple samples must be investigated to evaluate properly the malignant character of renal cell carcinoma" [5]. Early investigations into the metastatic heterogeneity of RCC also involved the development of a nude mouse model for evaluating RCC metastasis [6]. Employing this mouse model, Fidler and colleagues used the SN12C RCC line which had a heterogeneous subpopulations of cells with varied metastatic potential, as well as cells derived from spontaneous lung metastases [6]. These investigations provided some early tools to individually study RCC variants with high metastatic potential and to develop models for dissecting tumor evolution and metastasis [7].

More recently, advances in molecular phenotyping techniques such as next-generation sequencing have allowed for a deeper understanding of RCC evolutionary biology through the detection of genetically distinct subclones within individual tumors and the characterization of clonal architecture [8]. This technology has subsequently been used to study intratumor heterogeneity not just in RCC but in a diverse range of tumor types including breast cancer [9, 10], pancreatic cancer [11], ovarian carcinoma [11], and acute leukemia, [13–15], among others. This chapter will summarize recent data that employed modern molecular techniques to shed light on RCC heterogeneity, clonal evolution, and the potential clinical implications of these findings.

5.2 Intratumor Heterogeneity

Employing whole-exome sequencing to study intratumoral heterogeneity, Gerlinger et al. analyzed multiple regions from ten primary tumors and their associated metastases in three cases [16, 17]. These investigators found that 67 % of identified somatic mutations were heterogeneous and not detectable across all sampled regions within an individual tumor. Mutational intratumoral heterogeneity was seen for multiple tumor suppressor genes converging on loss of function. In addition, these investigators applied a 110-gene signature shown to classify ccRCC into good prognostic and poor prognostic molecular subgroups on spatially distinct regions of one tumor sample. The metastatic tumors and one region of the primary tumor segregated into the good prognostic subgroup, while the remaining regions of the primary tumor segregated into the poor prognostic subgroup, further illustrating the significant molecular heterogeneity within an individual tumor.

Martinez et al. [18] further characterized the extent of intratumoral heterogeneity by comparing individual tumor samples of clear cell RCC with unrelated tumor samples collected from the Cancer Genome Atlas (TCGA). Twenty-five percent of tumor biopsies demonstrated greater genetic similarity with unrelated tumor samples than with samples originating from the same primary tumor.

To further assess intratumoral genetics that underlie the mutational spectrum of clear cell RCC, Xu et al. performed single-cell exome sequencing using material from a kidney cancer and its adjacent normal kidney tissue [19]. These investigations revealed that the kidney tumor was unlikely to have evolved from mutations in VHL and PBRM1. Quantitative population genetic analysis interestingly showed that the tumor did not contain any significant clonal subpopulations. However, this analysis revealed that mutations with different allele frequencies within the population had different mutational spectra, suggesting that clear cell RCC "may be more genetically complex than previously thought" [19]. Novel algorithms to construct phylogenetic models of tumor progression at the cellular level – incorporating copy number changes at the scale of single genes, entire chromosomes, and the whole genome – are currently under development and may help shed additional light on the implications of single-cell sequencing [20].

5.3 Heterogeneity in Variant RCC Histologies

Investigations of RCC heterogeneity extend beyond that of clear cell histology into that of less common variant subtypes. Using next-generation sequencing (NGS), Durinck et al. analyzed exome, transcriptome, and copy number alteration data from 167 primary human tumors that included renal oncocytomas and non-clear cell RCC consisting of papillary (pRCC), chromophobe (chRCC), and translocation (tRCC) subtypes [21]. Within the non-clear cell subtypes, these investigators found that pRCCs had a higher mutation rate than chRCCs and renal oncocytomas and that genes altered in non-clear cell RCC were distinct from that reported with clear cell histology. Ten significantly mutated genes were identified in pRCC, including MET, NF2, SLC5A3, PNKD, and CPQ. In chRCC, the following genes were found to be significantly mutated: TP53, PTEN, FAAH2, PDHB, PDXDC1, and ZNF765. Interestingly, gene expression analysis identified a five-gene set that molecularly classified chRCC, renal oncocytoma, and pRCC.

Malouf et al. described the genomic and epigenetic characteristics of translocation renal cell carcinoma (tRCC), a rare subtype of kidney cancer involving the TFEB/TFE3 genes [22]. These investigators reported moderate cytogenetic heterogeneity in this rare tumor type, with 31.2 % and 18.7 % of cases presenting similarities with clear cell and pRCC profiles, respectively. The most common alterations seen were 17q gain in 44 % and 9p loss in 37 %. Exome sequencing of tRCC revealed a distinct mutational spectrum with frequent mutations in chromatin-remodeling genes [23].

A study of molecular heterogeneity in RCC with sarcomatoid differentiation using X-chromosome inactivation analysis suggested that both clear cell and sarcomatoid components of renal cell carcinomas were derived from the same progenitor cell [24]. Additionally, different patterns of allelic loss in multiple chromosomal regions were reported in clear cell and sarcomatoid elements from the same patient, suggesting divergence during RCC clonal evolution.

5.4 Branching Evolution

Gerlinger et al. utilized genetic analyses to construct phylogenetic trees by inferring ancestral relationships of tumor subclones [17]. These phylogenetic trees of ccRCC demonstrated branched rather than linear evolutionary patterns in all ten samples analyzed. Early ubiquitous genetic alterations were mapped to the truncal portion of the phylogenetic trees, while later heterogeneous alterations occurring in separate spatial regions composed the branches. Known driver mutations of ccRCC were mapped onto the phylogenetic trees to determine whether specific

driver genes were predominantly altered on truncal or branch portions [25]. Alterations in the von Hippel-Lindau (VHL) tumor suppressor gene were identified ubiquitously on the truncal portions of each phylogenetic tree consistent with its role as a critical founder event in the pathogenesis of ccRCC. However, the majority of known driver mutations were mapped onto the branches of the phylogenetic trees with 73 % of driver mutations identified in subclonal populations. These mutations included alterations in PTEN, SETD2, KDM5C, PBRM1, and BAP1 expression identified in spatially separate subclones.

Tumor subclones frequently displayed evidence of convergent phenotypic evolution. Three distinct alterations of SETD2 were identified with different regional distributions in one patient tumor. Splice-site mutations were carried in one biopsy site, a missense mutation was identified in metastatic sites, and a two-base-pair frameshift deletion was detected in all other tumor sites. Convergent evolution was also observed for KDM5C, PIK3CA, BAP1, and PBRM1 with different disruptive mutations identified in regionally separate tumor sites.

5.5 Implications for Clinical Practice

The presence of significant intratumoral heterogeneity in RCC presents several challenges to clinical practice. In addition, the presence of branching evolution can influence biomarker identification and validation, evaluation of prognosis, and even therapy resistance [26]. Current therapeutic decision-making is frequently based on characteristics of a single tissue biopsy of a primary tumor or a metastatic site. The genetic profile of the biopsy is assumed to be uniformly expressed in all other sites of disease. The presence of intratumoral heterogeneity confounds this assumption and may lead clinicians to wonder whether multiple biopsies will be necessary to accurately characterize a tumor [27]. There are several barriers to performing multiple biopsies in a patient. Multiple procedures may be associated with significant physical and psychological morbidity, and access to metastatic sites may be technically difficult or impossible. In addition, it is unknown how many biopsies are necessary to accurately characterize a tumor. Gerlinger et al. attempted to identify the optimal number of biopsies to reliably detect the majority of somatic mutations in a tumor but reported that a persistent increase in the number of detected mutations was observed with each additional biopsy in a majority of cases [16]. This observation casts doubt on the assertion that multiple biopsy attempts can accurately characterize a patient's tumor. A different perspective was offered by Sankin et al. who obtained core needle biopsies from three to five different regions of resected renal tumors and performed targeted DNA sequencing on five genes associated with ccRCC (VHL, PBRM1, SETD2, BAP1, and KDM5C) [28]. These investigators estimated that sampling three different tumor regions was sufficient to detect mutations in PBRM1, SETD2, BAP1, and/or KDM5C with 90 % certainty but noted that the mutational burden of renal tumors varied by region sampled.

The branched evolutionary pattern of ccRCC genetic alterations also poses additional challenges to the clinician and to drug development. Somatic alterations that may be theoretically "actionable" may not be ubiquitously present in all tumor subclones and thus may represent an inadequate therapeutic target. To date, there are no therapeutic drugs that directly and fully address the consequences of VHL tumor suppressor inactivation, even though this alteration represents the only ubiquitous "truncal" event in ccRCC. The identification of intratumoral heterogeneity may spur additional research into the development of agents that can target "truncal" alterations or increase interest in combinatorial drug therapy that can target several subclonal driver mutations.

The detection of intratumoral heterogeneity in RCC also raises the question of whether improved biomarkers or detection modalities may be necessary to fully characterize this heterogeneity. We may end up determining that characterization of the dominant tumor subclone is sufficient for guiding clinical therapy. The development of technologies that detect circulating serum biomarkers such as free tumor DNA (cfDNA) may

hold potential to detect and characterize the dominant tumor subclone at a given time in therapy but will require further research and validation in RCC [29–31].

Additionally, intratumoral heterogeneity can also affect the pharmacodynamic properties of anticancer therapies. A recent review article noted that the concentration of many anticancer drugs in human solid tumors is low, with strong variation in different parts of the tumor [32]. This scenario mirrors the genetic heterogeneity discussed in detail above. There is strong likelihood that in some malignancies such as RCC, therapy resistance may result from insufficient and/or heterogeneous exposure of cancer cells to effective drug levels. More sensitive analytical methods to assess drug distribution within tumors coupled with novel noninvasive imaging techniques such as imaging mass spectrometry and fluorescence microscopy may allow for real-time drug localization in relation to the microscopic structure of the tumor. These newer techniques may provide insights into the relative contribution of tumor architecture on drug distribution [32].

Clinical Vignette

A 63-year-old female with medical history significant for hypertension and diabetes mellitus presented with dysuria, gross hematuria, and lower abdominal pain. Her primary care physician prescribed a course of oral antibiotics for a urinary tract infection, but symptoms persisted. A complete blood count showed anemia with a hemoglobin concentration of 9 g/dL. Computed tomography (CT) scan revealed a large 9 cm solid mass involving the lower pole of the left kidney, a 3 cm right liver lobe metastasis, and multiple 1–2 cm bilateral pulmonary metastases.

A preoperative assessment placed the patient at low risk for surgery. The patient underwent a left radical nephrectomy and was found to have clear cell renal cell cancer, Fuhrman grade 4. The patient is then referred to a medical oncologist.

Four weeks later, the patient is recovering well from surgery. She sees her medical oncologist who discusses treatment options including therapy with sunitinib. The patient agrees to therapy with sunitinib and begins a regimen of 50 mg orally once daily on a 4 weeks-on, 2 weeks-off schedule. Restaging CT scans performed after 12 weeks of total therapy reveal interval improvement in her bilateral pulmonary metastases that are now ranging from 0.5 to 1.5 cm in greatest dimension. However, her right liver lobe lesion has now increased to 4.6 cm in size. She asks her oncologist to explain to her why she appeared to have a good response to therapy in her lungs but not in her liver.

This case is an illustration of a common clinical scenario. This patient with metastatic renal cell cancer has developed a mixed response to therapy with antiangiogenic therapy. The tumor biology of the liver metastasis apparently differs from the pulmonary metastases. If advanced molecular profiling were performed on each metastatic tumor, differences in expression of key driver mutations may be discovered that explain the variable response to therapy.

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The Metabolic Basis of Kidney Cancer

6

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Contents

Key Points

- Studies of familial kidney cancer syndromes have identified key genetic and molecular alterations that serve as drivers in certain kidney tumors.
- Altered metabolism is a common theme underlying the different subtypes of RCC.
- The genes involved in familial kidney cancer syndromes modulate nutrient, oxygen, and energy-sensing mechanisms that allow for metabolic adaptation to the tumor microenvironment.
- Elucidation of the complex metabolic networks operating in RCC will inform future design of therapeutic strategies.

6.1 Introduction

An estimated 63,920 cases of kidney cancer were diagnosed in 2014 leading to 13,860 deaths from the disease [1]. Renal cell carcinoma represents approximately 95 % of

Disclosure of Funding This research was funded in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, and Center for Cancer Research, Bethesda, MD, USA.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_6

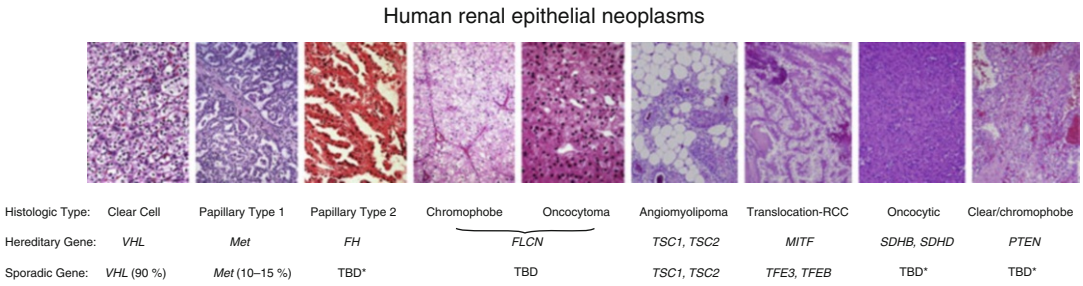

Fig. 6.1 Kidney cancers are a diverse group of malignancies with distinct genetic and molecular alterations and disparate histologic features (Permission granted from Elsevier) (Linehan et al.)

neoplasms arising from the kidney and is composed of a diverse group of malignancies with distinct genetic and molecular alterations, disparate histologic features, and unique clinical characteristics (Fig. 6.1). Despite this heterogeneity, targeted therapies for metastatic disease have centered on alterations in the von Hippel–Lindau (VHL)–hypoxia-inducible factor (HIF)–vascular endothelial growth factor (VEGF) pathway. While this approach is effective in clear cell Renal Cell Carcinoma (ccRCC), it has predictably met with limited success in patients with non-clear cell variants. The study of familial kidney cancer syndromes including von Hippel–Lindau (VHL) syndrome, hereditary papillary renal cell carcinoma (HPRC), hereditary leiomyomatosis and renal cell carcinoma (HLRCC), Birt–Hogg–Dubé syndrome (BHD), tuberous sclerosis complex (TSC), Cowden's disease, and succinate dehydrogenase renal cell carcinoma (SDH-RCC) has identified a number of genes involved in nutrient, oxygen, and energy-sensing mechanisms that allow for metabolic adaptation in the tumor microenvironment [2] (Fig. 6.2). As our understanding of cancer biology has evolved, so has our insight into divergent metabolic processes that appear to be critical for tumor proliferation and survival [3, 4]. Comprehending the roles of these altered metabolic pathways and exploiting the metabolic differences between normal and cancer cells will potentially aid in developing novel therapeutic and disease-specific targets.

6.2 Overview of Metabolism in Cancer

Proliferating cells require a steady source of nutrients to meet their demand for energy (ATP) and to serve as building blocks for macromolecules (lipids, proteins, nucleic acids, etc.) that are essential components of newly formed cells. Cancer cells are particularly adept at diverting available nutrients toward pathways conducive to their agenda of dysregulated proliferation [5]. This is accomplished, at least in part, by extensive reorganization of cellular metabolism to ensure (1) a constant supply of critical intermediates required for biosynthesis and (2) the generation of sufficient energy to fuel this process [6, 7]. While our understanding of the metabolic alterations in cancer cells is still evolving, considerable progress has been made toward this end in the past decade.

6.2.1 Glycolysis, the Krebs Cycle, and the Warburg Effect

Glucose is both the primary source of cellular ATP and a major contributor of carbon for macromolecule synthesis. Mammalian cells convert glucose into pyruvate in a multistep process referred to as glycolysis. In normal cells, in the presence of adequate oxygen, pyruvate is diverted to the mitochondrial Krebs cycle (tricarboxylic acid cycle, TCA), leading to the generation of ATP via oxidative phosphorylation.

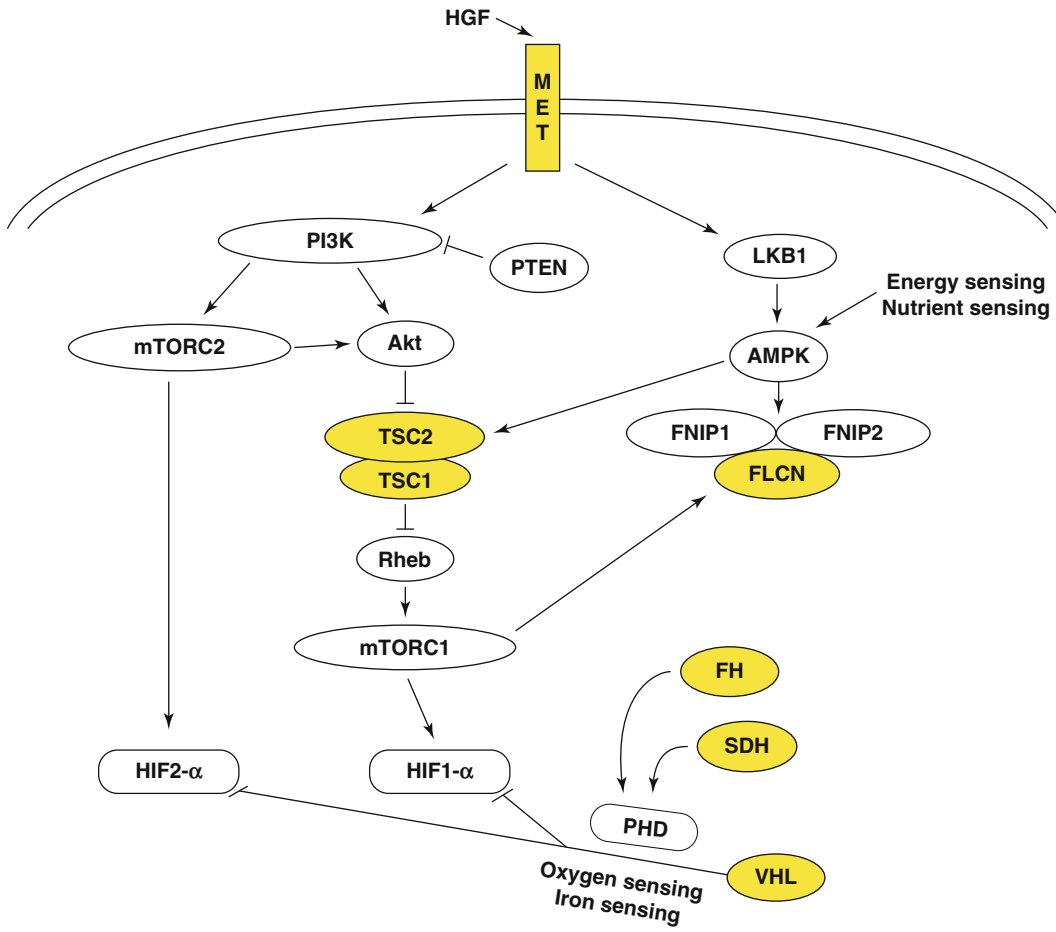

Fig. 6.2 The genes known to cause kidney cancer, *VHL*, *MET*, *FLCN*, *FH*, *SDH*, *TSC1*, and *TSC2* share the common feature in that each is involved in various pathways that regulate cell growth, proliferation, or nutrient metabolism pathways (Permission granted from Elsevier) (Linehan et al.)

However, under anaerobic conditions, ATP is generated by a process that results in conversion of pyruvate to lactate in the cytoplasm. While the latter process is able to meet cellular bioenergetic requirements as long as the availability of glucose is not limiting, it is a far less efficient means of generating ATP than is oxidative phosphorylation. In addition to its essential role in ATP synthesis, the Krebs cycle also plays an important role in macromolecule synthesis with intermediates such as citrate, oxaloacetate, and α-ketoglutarate serving as critical components of biosynthetic pathways leading to generation of lipids, nucleotides, and proteins.

The concept of altered metabolism in cancer is not new; as early as the 1920s, the German scientist and subsequent Nobel laureate Otto Warburg noted that compared to normal cells, tumor cells (derived from ascitic fluid in his original experiments) consume larger amounts of glucose and generate excessive amounts of lactic acid. Based on these observations, he concluded that cancer cells utilize glycolysis preferentially as a means of ATP synthesis (i.e., aerobic glycolysis or the "Warburg Effect") [8, 9]. He further posited that this phenomenon was the result of an intrinsic defect in mitochondrial function and oxidative phosphorylation, forcing cells to turn to aerobic

glycolysis. Although there is overwhelming evidence of the Warburg effect in a wide range of cancers, Warburg's suggestion that this results from mitochondrial dysfunction is supported only in a small minority of tumors, including at least two subsets of kidney cancer [10, 11]. Instead, in most cancers where the Warburg effect is evident, the reliance on aerobic glycolysis for ATP generation appears to be an attempt to spare Krebs cycle intermediates for preferential use as substrates for biosynthetic pathways [12]. Regardless, aerobic glycolysis appears to be essential for tumor proliferation and survival in many human cancer models and is a valid and promising target for therapeutic intervention.

6.2.2 Drivers of the Warburg Effect in Human Cancer

The precise mechanisms that promote aerobic glycolysis in cancer cells are a matter of ongoing debate. It is becoming increasingly clear that there is no single unifying mechanism driving the glycolytic shift across all cancers, with distinct processes identified in disparate tumor types. Activation of several oncogenes as well as inactivation of a variety of tumor suppressor genes has been implicated in promoting the glycolytic phenotype. Inactivation of the Krebs cycle enzymes fumarate hydratase or succinate dehydrogenase in some forms of familial kidney cancer offers the best examples of direct interruption of oxidative phosphorylation and obligate use of aerobic glycolysis for ATP generation [10, 11]. The PI3K/Akt/mTOR pathway is an important regulator of cell growth and proliferation and plays a key role in nutrient sensing and cellular responses to growth factors [13]. Activation of this pathway has been described in several cancers and can lead to upregulation of glycolysis by several mechanisms, including increased influx of glucose and other nutrients, transcriptional activation of key glycolytic enzymes, and enhanced translation of a number of proteins essential to the process [14, 15]. Similarly, amplification of the proto-oncogene *MYC* has been shown to activate glycolysis via upregulation of enzymes regulating this process (including PKM-2, hexokinase, and LDH-A) as well as upregulation of transmembrane glucose transporters such as GLUT-1 [16, 17]. Activation of the NRF2 oxidative stress response pathway is a feature of several cancers including non-small cell lung cancer and some forms of papillary RCC; recent evidence suggests that this pathway promotes metabolic reprogramming for cancer cells by enhancing glycolysis as well as glutaminolysis [18, 19].

Hypoxia-inducible factor-1 (HIF-1) is an important component of the cellular oxygen-sensing apparatus and plays a key role in the regulation of glycolysis in response to hypoxia. In normoxic conditions, key proline residues in the alpha subunit of HIF-1 are hydroxylated by prolyl hydroxylase. The *VHL* gene encodes the VHL protein which binds to hydroxylated HIF-1α, targeting the latter for ubiquitin-mediated degradation [20, 21]. In hypoxic conditions, prolyl hydroxylase is inhibited, permitting an HIF-1α to accumulate. Stabilization of HIF-1α enables the cell to respond to hypoxic and low-iron conditions by transcriptional upregulation of a number of genes critical for cancer proliferation and activation of aerobic glycolysis including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), glucose transporter 1 (GLUT1), phosphofructokinase-2 (PFK2), and pyruvate dehydrogenase (PDH). In many human cancers, HIF-1α is stabilized by a variety of mechanisms independent of ambient oxygen tension. One of the best studied mechanisms mediating this pseudohypoxic HIF response is seen in VHL-deficient tumors. Due to VHL inactivation in these tumors, HIF-1α is no longer appropriately targeted for ubiquitin-mediated degradation even when oxygen is readily available, a phenomenon that is seen in both VHL-associated and sporadic ccRCC [22]. VHL-independent upregulation of HIF-1 has also been described, particularly in RCC variants where disruption of the Krebs cycle leads to accumulation of substrates such as fumarate and succinate, which impede the hydroxylation of the alpha subunits of HIF-1 [11, 23]. Additionally, translational upregulation of HIF-1α has been described with

activation of the mammalian target of rapamycin (mTOR) signaling, which can occur by a variety of mechanisms [24, 25].

6.2.3 Glutaminolysis and Reductive Carboxylation

Glutamine is an additional important nutrient substrate in tumor cells, contributing to the generation of citrate and acetyl coenzyme A for lipid synthesis as well as acting as a nitrogen donor for amino acid/protein synthesis. A key step in glutamine metabolism is its deamidation by glutaminases to generate glutamate. While this reaction is bidirectional in normal cells, in tumor cells, deamidation of glutamine to glutamate is favored, partly a result of the overexpression of glutaminases and suppression of glutamine synthetase [26, 27]. Glutamate is converted to α-ketoglutarate which enters the Krebs cycle and can be converted to oxaloacetate in one of two ways. In tumors with an intact Krebs cycle and electron transport chain, mitochondrial oxidative metabolism is the predominant pathway used. However, in instances where oxidative metabolism is impaired (e.g., mutations in *FH* or *SDH*), an alternative pathway of reductive carboxylation is employed to generate both oxaloacetate and acetyl CoA [28]. In the latter instances, glutamine is the major carbon source for fatty acid synthesis [28]. Glutaminolysis also allows tumor cells to meet the NADPH demands of growth and supports enhanced fatty acid synthesis, nucleotide biosynthesis, and maintenance of the glutathione pool [12].

6.3 Metabolic Alterations in Kidney Cancer

Although metabolic reprogramming is a common theme in the different subtypes of kidney cancer, there are significant differences in the nature of the metabolic alterations and the genetic and molecular mechanisms driving these alterations between individual RCC variants. Elucidating the precise changes underlying each entity is crucial for developing specific targeting strategies against a given subtype.

6.3.1 Kidney Cancers Harboring Mutations in Krebs Cycle Enzyme Genes

Inactivating germ line mutations or deletions of *fumarate hydratase* and subunits of *succinate dehydrogenase* are associated with distinct forms of RCC that offer classic examples of the Warburg effect in human cancer. In both cases, the basic underlying defect is an enzymatic deficiency that impairs the Krebs cycle and oxidative phosphorylation, with a consequent glycolytic shift.

6.3.1.1 Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC)

HLRCC is an autosomal dominant hereditary cancer syndrome characterized by the presence of potentially painful cutaneous leiomyomas occurring on the arms or trunk and development of early-onset uterine fibroids in affected women (often necessitating hysterectomy in the third or fourth decade of life) [29]. In addition, affected patients are at risk for developing a particularly aggressive form of renal cancer with potential for rapid growth and early metastasis [30]. HLRCC-associated kidney cancer presents as early-onset, unifocal or bilateral, multifocal renal cysts or type 2 papillary tumors that have a characteristic histologic appearance that differentiates it from other forms of RCC [31]. Localized renal tumors are managed surgically, with even small tumors being removed due to a heightened risk of metastases. Once metastatic, this form of kidney cancer is almost always fatal.

The underlying genetic alteration is a germ line mutation/deletion of the *FH* gene; a "second hit," i.e., loss of the normal somatic allele, precedes development of kidney cancer. Inactivation of *FH* has several consequences. Loss of fumarate hydratase activity leads to accumulation of its substrate, fumarate, interrupting a key oxidative metabolic pathway critical for meeting cellular bioenergetic and biosynthetic needs. Impairment of the Krebs cycle imposes a metabolic shift characterized by a reliance on aerobic glycolysis for generating ATP and by the use of glutamine as the major source of carbon for fatty acid synthesis.

The metabolic shift in FH–/– cells is initiated and sustained by a number of factors. First, excess fumarate inhibits hydroxylation of HIF-1α, leading to the accumulation of this key driver of aerobic glycolysis. Additionally, FH–/– cells are characterized by decreased activity of activated protein kinase adenosine monophosphate kinase (AMPK), the master energy sensor of the cell, with consequent upregulation of mTOR activity [32]. Decreased AMPK activity also leads to decreased cytosolic iron levels by downregulating iron transporter activity (DMT1), which further diminishes the catalytic activity of prolyl hydroxylases, complementing the competitive inhibition of these enzymes imposed by fumarate accumulation. Lastly, excess fumarate leads to activation of an oxidative stress response mediated by nuclear factor E2-related factor 2 (Nrf2) [33]. Nrf2 activity is normally regulated tightly by an E3 ubiquitin ligase complex composed of Kelch-like ECH-associated protein 1(KEAP1) and cullin 3 (cul3). In FH–/– cells, KEAP1 undergoes a post-translational modification (succination at cysteine residues) disrupting its interaction with Nrf2, thus allowing the latter to accumulate in the nucleus. Nrf2 is an essential transcriptional regulator mediating the cellular antioxidant response which is critical for neutralizing the effect of reactive oxygen species during oxidative stress. Activation of Nrf2 has been shown to redirect glucose and glutamine into anabolic pathways including glycolysis and the pentose phosphate pathway. The Nrf2 pathway is also activated in some forms of sporadic papillary RCC; mutations in KEAP1, Nrf2, and cul3 leading to disruption of protein–protein interactions have been described and are thought to mediate the oxidative stress response in these tumors [34]. The reliance of FH–/– cells on glycolysis and glutamine catabolism offers rational therapeutic targets. Approaches directed against these pathways are subjects of ongoing preclinical and clinical investigation.

6.3.1.2 Succinate Dehydrogenase Deficiency Kidney Cancer

SDH is the only enzyme that participates in both the TCA cycle and the electron transport chain; SDH is composed of four subunits that are bound to the inner mitochondrial membrane. Germ line mutations in genes encoding the SDH-C, SDH-B, and SDH-D subunits are associated with hereditary paraganglioma of the head and neck in addition to hereditary pheochromocytoma [35, 36]. Alteration of these subunits has been found to be associated with familial kidney cancer that can present with or without paragangliomas or pheochromocytoma [37, 38]. Loss of SDH activity leads to disruption of the TCA cycle and accumulation of succinate, with many biochemical and metabolic consequences similar to those seen with FH inactivation. Elevated levels of succinate inhibit the activity of prolyl hydroxylases, preventing the hydroxylation of HIF with resultant accumulation [11]. SDH-RCC presents with an aggressive phenotype associated with early onset and presentation with local symptoms or systemic manifestations associated with metastatic disease [39]. Treatment strategies similar to those employed in HLRCC are under investigation.

6.3.2 Von Hippel–Lindau Syndrome and Clear Cell Renal Cell Carcinoma

Patients with VHL syndrome present with a constellation of manifestations including central nervous system hemangioblastomas (cerebellum and spine), retinal angiomas, endolymphatic sac tumors of the inner ear, pheochromocytoma, pancreatic serous cystadenomas, neuroendocrine tumors, and epididymal cystadenomas, as well as renal cysts and clear cell kidney cancer. VHL patients often develop numerous, bilateral renal cysts, solid tumors, and mixed lesions. Renal tumors in VHL patients rarely metastasize when less than 3 cm in greatest dimension. Active surveillance of these cancers until they reach this size threshold has allowed for renal preservation while minimizing the number of surgical interventions needed to maintain oncologic outcomes [40, 41].

Genetic linkage analysis in VHL families led to the identification of the *VHL* tumor suppressor gene, located on the short arm of chromosome 3 [42, 43]. As a well-studied hereditary kidney cancer syndrome, it has provided profound insight

into the behavior of both VHL-associated tumors and of nonfamilial clear cell kidney cancer. Indeed, somatic VHL gene alterations have been identified in up to 90 % of patients with sporadic ccRCC [44]. The VHL protein product is a component of an E3 ligase that targets several proteins for ubiquitin-mediated degradation [20]. The most widely studied consequence of VHL inactivation is its effect on the alpha subunits of the hypoxia-inducible factors. Loss of functional VHL protein prevents the ubiquitin-mediated degradation of HIF-α, leading to intracellular accumulation of HIFs [21, 22]. The stabilization of HIFs independent of ambient oxygen tension leads to the transcriptional activation of a number of genes including those encoding proangiogenic factors (VEGF, PDGF), glucose transporters (GLUT1), and growth factors (TGF-α/EGFR) which are necessary for tumor growth and survival. Elucidation of the critical role played by dysregulated VHL/HIF activity in the genesis of ccRCC has been instrumental in the development of clinically effective targeted strategies in this disease; antagonists of the VEGF pathway are currently a mainstay of therapy for patients with metastatic ccRCC.

The metabolic alterations underlying ccRCC are not as well characterized as those in tumors with impaired Krebs cycle activity. It is, however, clear that ccRCC is characterized by several features that promote a glycolytic phenotype. HIF-1 is often upregulated in VHL-deficient ccRCC, usually in conjunction with an activated Akt/mTOR pathway. The Cancer Genome Atlas recently conducted a comprehensive molecular characterization of over 400 primary ccRCC tumors [45]. In addition to VHL mutations, activation of the PI3K/Akt pathway was a recurrent theme, as was amplification of *MYC*. This analysis also uncovered evidence of metabolic reprogramming reminiscent of the Warburg effect, particularly in high-grade/high-stage tumors associated with poor survival; Krebs cycle enzymes and AMPK were downregulated, while glycolytic enzymes and enzymes catalyzing lipid synthesis were upregulated in these tumors. While attempts to target metabolic aberrations in ccRCC are still in the early stages of development, it is notable that patients with poor prognosis appear to derive clinical benefit from temsirolimus, an inhibitor of mTOR activity. Given the central role played by mTOR in regulating cellular metabolism, it is tempting to speculate that the activity of mTOR inhibitors in this context might relate to their ability to reverse the altered metabolic phenotype.

6.3.3 Hereditary Papillary Renal Cell Carcinoma and Type 1 Papillary Renal Cell Carcinoma

HPRC is an autosomal dominant condition resulting from activating mutations of the *MET* proto-oncogene located on 7q31. Affected individuals are at risk for developing bilateral, multifocal type 1 papillary RCC. Somatic *MET* mutations are seen in approximately 10–15 % of papillary kidney cancers; additionally, chromosome 7, on which both MET and its ligand HGF are located, is duplicated in a significant percentage of papillary RCC, and it has been suggested that this may represent an alternative means of activating the HGF/MET pathway [46].

MET encodes the hepatocyte growth factor receptor which exhibits tyrosine kinase activity [47]. In normal cells, MET is activated by binding to HGF; however, mutations in the tyrosine kinase domain, as seen in HPRC, render MET constitutionally active. Activation of MET engages multiple signal transduction pathways including the phosphatidylinositol 3-kinase pathway (PI3K). PI3K-RAS and PI3K-AKT activation play a role in increased expression of nutrient transporters resulting in additional uptake of glucose and amino acids. Mutations leading to constitutive activation of HGF/MET can also overcome the negative regulation of AMPK in response to nutrient starvation and/or low cellular ATP (which in normal conditions will promote fatty acid oxidation and ketogenesis).

Clinical management of HPRC is similar to that of ccRCC in VHL syndrome, with active surveillance of lesions until growth of at least one tumor to 3 cm triggers nephron-sparing surgical intervention. A small molecule kinase inhibitor of MET and VEGF receptors (Foretinib) demonstrated an

overall response rate of 13.5 %, with the presence of a germ line *MET* mutation being highly predictive of response (50 % response rate in those with activating germ line *MET* mutations) [48].

6.3.4 Birt–Hogg–Dubé Syndrome and Chromophobe Renal Cell Carcinoma

Germ line mutations of the tumor suppressor *FLCN,* located on the short arm of chromosome 17 at position 11.2, have been noted in a large proportion of Birt–Hogg–Dubé (BHD) families with up to 70 % of BHD-associated tumors demonstrating loss of heterozygosity or sequence alterations in the somatic copy of *FLCN* [49, 50]. BHD is inherited in an autosomal dominant fashion and can be associated with pulmonary cysts predisposing to spontaneous pneumothorax, fibrofolliculomas, and renal tumors including chromophobe, oncocytic, and clear cell RCC in addition to oncocytomas and hybrid chromophobe/oncocytic tumors.

Folliculin, the protein product of *FLCN*, interacts with two other cellular proteins, folliculin interacting proteins 1 and 2 (FNIP1 and FNIP2), which aid in folliculin localization to lysosomes during amino acid starvation [51]. FNIP1 and FNIP2 are both phosphorylated by AMPK and bind to the γ-subunit of AMPK which responds to low levels of ATP and nutrients by inhibiting mTOR activity and thereby downregulating cellular metabolism, protein synthesis, and growth [52]. Both in vitro studies and animal models of FLCN inactivation suggest that loss of folliculin is associated with activation of both mTORC1 and mTORC2 activity [26]. The metabolic changes resulting from FLCN loss are yet to be fully characterized. Despite the presence of increased mTOR activity, the preponderance of evidence suggests that oxidative phosphorylation is active in BHD-associated tumors. In a mouse model where FLCN was selectively knocked out in skeletal muscle, FLCN-null cells appeared to have increased mitochondrial activity and demonstrated a shift toward oxidative phosphorylation [53]. The metabolic environment in BHD-associated tumors is likely to be more complex than simply a preference for oxidative phosphorylation and is the subject of ongoing investigation. A sporadic counterpart of BHD-associated tumors has not yet been identified. However, sporadic forms of chromophobe RCC (one of the histologic subtypes seen in BHD patients) appear to be characterized by genetic alterations that lead to increased mTOR activity (including PTEN loss, inactivating mutations in TSC1/2, and activating mTOR mutations) [54]. Additionally, mutations in mitochondrial genes that may promote a glycolytic phenotype have also been identified in these tumors, although their precise role in tumorigenesis and metabolic regulation remains to be determined [44].

6.3.5 Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder resulting from germ line mutations in the TSC1 or TSC2 genes which act as tumor suppressor genes. Affected individuals are at risk for involvement of multiple organs, and CNS, skin, pulmonary, cardiac, ocular, and renal manifestations have been described. Although the most common renal manifestation is the presence of angiomyolipomas, clear cell RCC is not uncommon [55].

Tuberin and hamartin, the protein products of TSC1 and TSC2, respectively, form a complex with a GTPase-activating protein complex which acts to inhibit mTOR activity [56]. TSC loss is associated with increase in mTORC1 activity and elevated HIF-1α levels; in vitro, treatment of TSC2-deficient cells with rapamycin has been shown to restore normal levels of HIF, supporting the role of TSC2 in regulation of HIF translation by mTOR inhibition [57].

6.3.6 TFE-Fusion Renal Cell Carcinoma

Transcription factor E (TFE)3 and TFEB are transcription factors that belong to the microphthalmia-associated (MiT) family of transcription factors. Gene translocations involving both TFE3 and

TFEB have been described in a variant of kidney cancer commonly referred to as "translocation RCC" characterized histologically by the presence of papillary and clear cell features and comprising around 1–5 % of all kidney cancers. TFE3 and TFEB are located on chromosome Xp11.2 and 6p21.2, respectively. A number of fusion partners for these transcription factors have been identified in kidney cancer (*PRCC–TFE3*, *ASPSCR1–TFE3*, *SFPQ–TFE3*, *NONO–TFE3*, *CLTC–TFE3*, and *MALAT1–TFEB*). Unique characteristics of this group include its predilection for presenting earlier in life, with up to 15 % of those with kidney tumors less than 45 years of age possessing a TFE gene fusion [58, 59]. A recent study suggests that these tumors may have identifiable imaging characteristics although these findings should be validated in a larger study [60–62].

TFE3-fusion cancers are usually aggressive and often present with advanced disease with an increased incidence of regional nodal involvement [63]. Recently, a germ line missense mutation in MiTF, another member of the family, has been shown to increase the risk of RCC in addition to a predisposition to melanoma [64].

The MiT transcription factor family has been implicated in a host of functions, notably cellular differentiation. TFE3 appears to be involved in diverse signaling pathways, but the precise role of individual pathways in the genesis of translocation RCC remains unclear. TFE3-fusion cancers have higher levels of phosphorylated S6, a downstream target of MTORC1 and marker of mTOR activation [65]. TFE3 also plays a role in insulin signaling and glucose metabolism via upregulation of the IRS-2 and hexokinase enzymes in the liver, although this role may be tissue specific [66]. Furthermore, TFEB is a master gene for lysosomal biosynthesis with a role in extracellular nutrient sensing and autophagy.

6.3.7 Cowden's Syndrome

Cowden's syndrome, or multiple hamartoma syndrome, is an autosomal dominant inherited disorder associated with mutations in the PTEN tumor suppressor gene. It presents with multisystem manifestations including benign trichilemmomas and acral keratosis of the skin, macrocephaly, intestinal hamartomas, and gangliocytoma of the cerebellum [67]. There is an increased risk of breast, thyroid, endometrial, and kidney cancer (30-fold increased risk according to some estimates) [68]. In one study, 4/24 patients with Cowden's syndrome had kidney cancer; the histology of RCC associated with this condition is varied, and papillary, chromophobe, and clear cell subtypes have all been described [69].

Loss of PTEN function results in constitutive activation of AKT, which in turn results in upregulation of mTOR. In animal models of PTEN loss, mice treated with rapamycin have demonstrated regression of disease-associated mucocutaneous lesions, and prolonged survival in those treated before appearance of disease manifestations [70].

6.4 Targeting Aberrant Metabolic Pathways in Kidney Cancer

It is now increasingly apparent that a variety of metabolic alterations accompanies malignant transformation and is essential for the growth and survival of tumors arising in the kidney. Recognition of the key role of metabolism in RCC has spurred significant interest in pharmacologically targeting metabolic pathways thought to be critical for tumor survival and proliferation. While there have been considerable advances in how we think about these targeted strategies, the field is still in its infancy.

It has been suggested that a number of enzymes mediating either glycolysis or glutaminolysis would be ideal targets. Indeed, a wide array of agents with purported activity against targets such as glucose transporters, hexokinase, PKM2, and glutaminase have been evaluated in preclinical models and early phase clinical trials with varying measures of success [71]. At least one agent targeting glutaminase, CB-839, is currently in phase 1 testing, with planned expansion cohorts once MTD is achieved, to evaluate its efficacy in a variety of solid tumors including RCC (NCT02071862). The identification of

AMPK as a key mediator of growth and metabolic signals in response to the nutrient and energy status of the cell has kindled interest in metformin, an activator of this molecule. Several trials of this agent, including phase 1 evaluation in combination with mTOR inhibitors in patients with solid tumors, are currently underway. The mTOR inhibitors temsirolimus and everolimus have activity in clear cell and other forms of RCC and are approved by the US FDA for treatment of patients with advanced kidney cancer. Given the importance of mTOR in regulating the metabolic phenotype in many forms of RCC, it is conceivable that the activity of agents targeting mTOR is at least partly a result of metabolic perturbations. The combination of bevacizumab and erlotinib has shown considerable activity in HLRCC-associated kidney cancer in a phase 2 study and is being explored further in this disease and in patients with sporadic papillary RCC [72]. The regimen was designed to exploit the dependence of these tumors on a high glucose flux to sustain aerobic glycolysis; it was hypothesized that the antiangiogenic properties of bevacizumab would limit glucose delivery to the tumor microenvironment while erlotinib might interfere with active glucose transport into the cells.

6.5 Summary

Over the past two decades, the complex heterogeneity underlying cancers arising in the kidney has become increasingly apparent. By studying familial kidney cancer syndromes, a variety of genetic and molecular alterations have been identified as key drivers in these tumors. As with many other malignancies, altered metabolism appears to be a common theme underlying the different subtypes of RCC. Although significant advances have been made in our ability to manage more common forms of kidney cancer, durable responses and cures remain elusive. It is hoped that elucidation of the complex metabolic networks operating in these tumors will inform future design of therapeutic strategies and help consolidate current gains in the battle against kidney cancer.

Clinical Vignette

A 58-year-old man presented with fatigue, anorexia, and 20 lbs weight loss over the past 12 weeks. His family history was significant for metastatic renal cell carcinoma in his maternal grandfather and mother who died at 55 and 47 years old, respectively, from the disease. He had multiple firm, painful, flesh-colored lesions noted on the trunk consistent with cutaneous leiomyomata (Fig. 6.3a). Genetic analysis revealed a germ line mutation in the *fumarate hydratase* gene. CT scans and fluorodeoxyglucose (FDG) PET/CT imaging revealed para-aortic and mediastinal lymphadenopathy as well as left para-psoas masses. The patient was enrolled on a phase 2 trial of bevacizumab in combination with erlotinib; a regimen devised to test the hypothesis that limiting glucose delivery to the tumor microenvironment and restricting cellular glucose uptake might be associated with clinical activity in patients with HLRCC-associated kidney cancer. Restaging studies obtained 8 weeks and 16 weeks following initiation of therapy reveal significant tumor regression at all metastatic sites consistent with a confirmed partial response (RECIST 1.1). He has tolerated therapy well with minimal side effects and continues to have a partial response 68 weeks following the initiation of therapy (Fig. 6.3b, c).

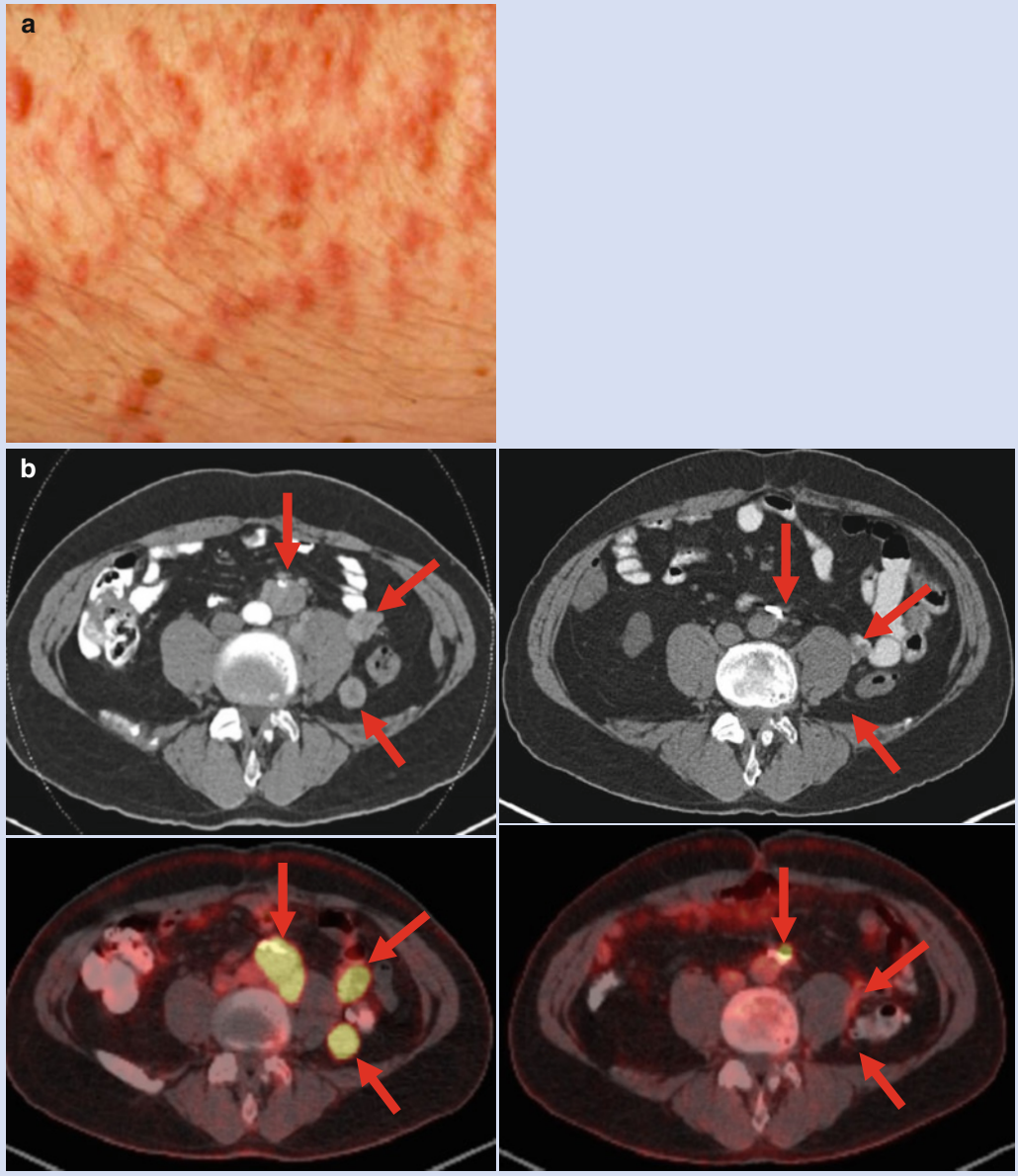

Fig. 6.3 (**a**) Representative images of multiple painful, flesh-colored nodules (cutaneous leiomyomas) 0.4–2.5 mm in size present on the extremities. (**b**) Abdominal CT scan and FDG PET/CT fusion images at baseline (*left panels*) and 16 weeks after initiation of bevacizumab and erlotinib (*right panels*) reveal significant regression of bulky retroperitoneal lymphadenopathy and metastases to soft tissue (*red arrows*). (**c**) Whole body FDG PET imaging at baseline (*left panel*) and 16 weeks after initiation of bevacizumab and erlotinib (*right panel*) reveal significant regression of FDG avid metastatic disease at multiple sites

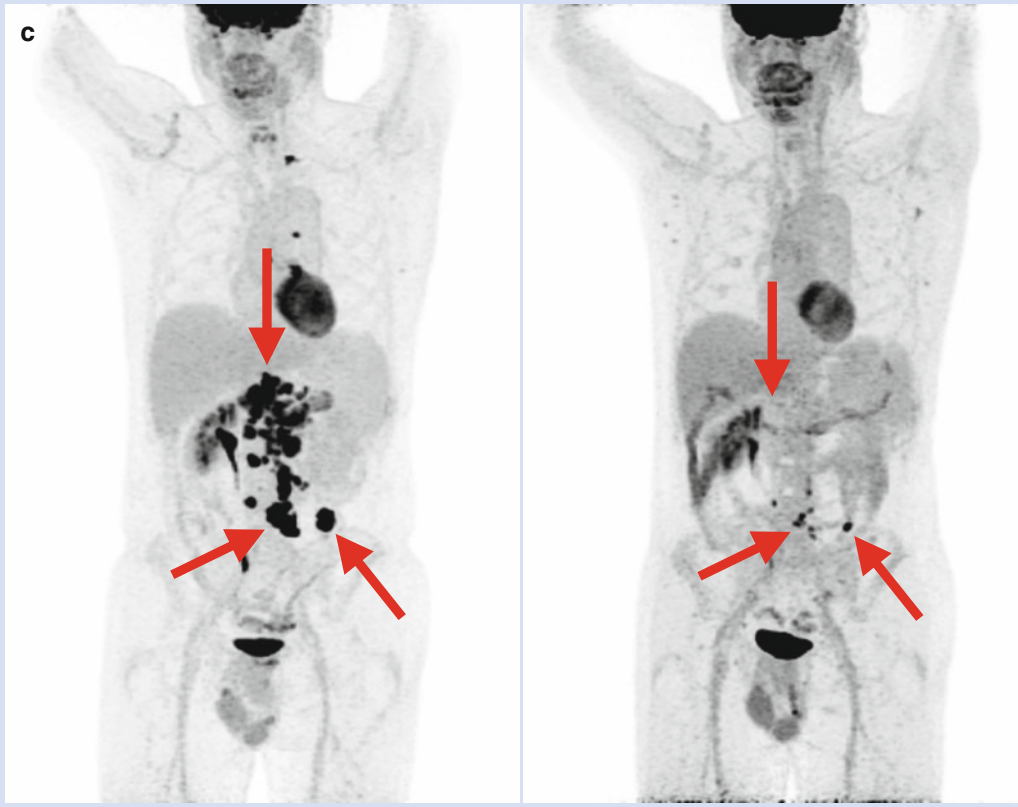

Fig. 6.3 (continued)

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Part II

Clinical Considerations

Renal Cell Carcinoma: Clinical Presentation, Staging, and Prognostic Factors

7

Sri Bharathi Yadlapalli, Dongping Shi, and Ulka Vaishampayan

Contents

Key Points

- Renal cancer is the tenth leading cause of cancer death in the United States.
- For localized tumors, the 5-year survival rate exceeds 85 %; however, this falls to 20 % or less for advanced or metastatic tumors. Unfortunately, approximately 25–30 % of patients with RCC present with metastatic disease.
- The critical gene involved in the pathogenesis of RCC is the von Hippel-Lindau tumor suppressor gene (*VHL*).
- Clear cell histology accounts for 70 % of renal cancers and is the most aggressive form. Chromophobe and papillary are indolent and minimally symptomatic at presentation.
- The most common paraneoplastic manifestations are hypertension and hypercalcemia.
- Ultrasound is often the first imaging modality used to evaluate patients with suspected RCC, but the gold standard for diagnosis, staging, and surveillance is the computed tomography scan.
- The staging system that is commonly employed is TNM system. Stage remains among the most important prognostic factors for the clinical behavior and outcome of RCC.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_7

- Several prognostic nomograms have been developed using clinicopathological features to predict patient outcome independent of treatment received.

7.1 Clinical Presentation

7.1.1 Symptoms and Signs (See Table 7.1)

The classic triad described in RCC is comprised of hematuria, flank pain, and fever but is seen in only 9 % of patients. Clinical presentation is actually extremely variable and is highly dependent on stage of presentation. The sequestered location of the kidney, in the retroperitoneum, results in asymptomatic and non-palpable masses that present only at an advanced or metastatic stage. Incidental detection has increased over time. Between 1935 and 1965, 7 % of tumors were discovered incidentally. In a National Cancer Institute (NCI) study conducted in metropolitan Detroit and Chicago from 2002 to 2007, the proportion of asymptomatic cases increased from 35 % in 2002 to 50 % in 2007. Cases before 1973 were found without the benefit of computed tomography (CT) or ultrasound scanning, whereas those after 1980 were discovered largely because of the widespread use of these technologies. What was once an internist's tumor has transformed into a radiologist's tumor. Incidental tumors diagnosed at an earlier stage obviously have a better prognosis. In a recent single-institution study, patients who underwent surgical resection from January 1, 1988, and December 31, 2007, were reviewed. Data were divided into four periods, with each time period encompassing 5 years. Over time the rate of incidental detection increased, from 10.6 to 27.6 % [1] largely because of imaging for evaluation of vague abdominal symptoms (see Fig. 7.1). The incidental tumors are more likely to be smaller (<4 cm) and have a lower grade (Fuhrman grade 1–2), which contributes to better cancer-related prognosis. The incidental finding of renal mass can also induce morbidity and the risks vs. benefits of therapy should be carefully considered, especially in cases with significant comorbidities (Table 7.2).

Table 7.1 RCC clinical presentation: symptoms and signs reported in different studies

Symptoms or signs	Skinner $n=309$ (%)	Gibbons $n=110$ (%)	Jayson $N=131$ (%)	Gupta $N=811$ group 1	group 4
Hematuria	59	37	24	29	**39**
Abdominal or flank mass	45	21	8	49	**22**
Pain	41	21	10	28	**13**
Weight loss	28	30		25	**16**
Symptoms from metastasis	10	–	–	–	–
Classic triad	9	–	–	–	–
Acute varicocele	2	–	–	–	–
Incidental finding	**7**	**40**	**61**	–	–

Approximately 25–30 % of patients with RCC present with locally advanced or metastatic disease. Expectedly, these patients can present with symptoms secondary to metastasis to distant sites. The most common sites of metastasis include:

- Lung: 50–60 % (Fig. 7.2)
- Bone: 30–40 % (Fig. 7.3)
- Liver: 30–40 %
- Soft tissue: 35 %
- Central nervous system: 8 %
- Cutaneous: 8 %

Depending on the organ involved, patients can present with hemoptysis, pleural effusion, cough, bone pain, back pain, pathological fracture, mental status changes, and headache.

Histology also appears to influence the initial clinical presentation. Clear cell RCC has a propensity for vascular invasion and is associated with distant metastasis at an early stage as compared to the papillary tumors that tend to have locoregional invasion with lymph node spread. Due to the low potential for early vascular invasion of papillary and chromophobe cancers distant metastases typically occur later in the disease course.

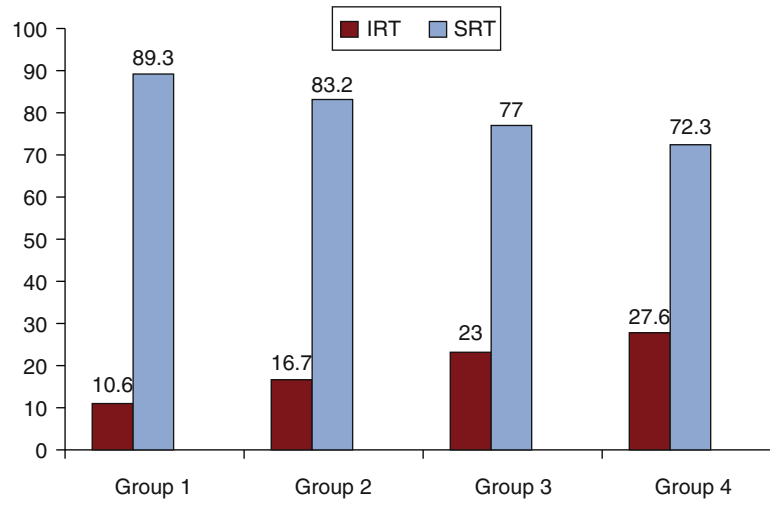

Fig. 7.1 Comparison of incidental and symptomatic presentation of RCC from different time periods. Group 1:1988–1992; Group 2:1993–1997; Group 3:1998–2002; Group 4: 2003–2007 (Reprinted with permission. Medknow Publications & Media Pvt. Ltd. All rights reserved. Gupta et al. [1])

Table 7.2 Paraneoplastic manifestations of RCC: incidence and prognostic significance

Type	Incidence (%)	Prognostic significance
Endocrine		
Hypercalcemia	13–20	Unfavorable
Hypertension	40	–
Polycythemia	1–8	–
Stauffer's syndrome	3–20	Unfavorable
Elevated alkaline phosphatase	10	Unfavorable
Cushing syndrome	2	–
Thrombocytosis	–	Unfavorable
Cachexia	30	Unfavorable
Non-endocrine		
Amyloidosis	3–8	–
Anemia	20	Unfavorable
Neuromyopathy	3	–
Vasculopathy	–	–
Nephropathy	–	–
Fever	20	

7.1.2 Paraneoplastic Manifestations

Paraneoplastic syndromes are defined as a collection of symptoms and clinical signs that occur in cancer patients, remotely from the tumor location. These are the result of humoral substances produced by the cancer cells (such as calcitriol production by RCC) or benign tissues generating humoral factors in response to malignancy (such as clubbing) or via modulation of the immune system.

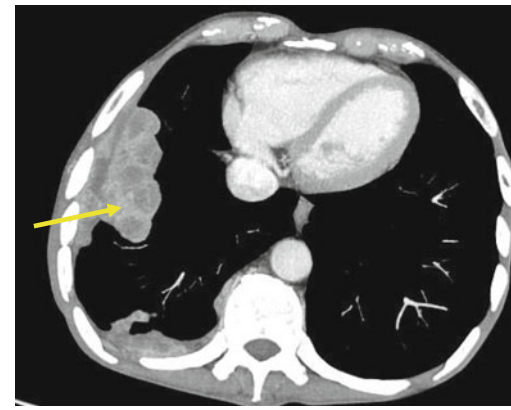

Fig. 7.2 Pulmonary metastasis in a patient with clear cell RCC. *Yellow arrow* shows sites of lung metastases from renal cancer

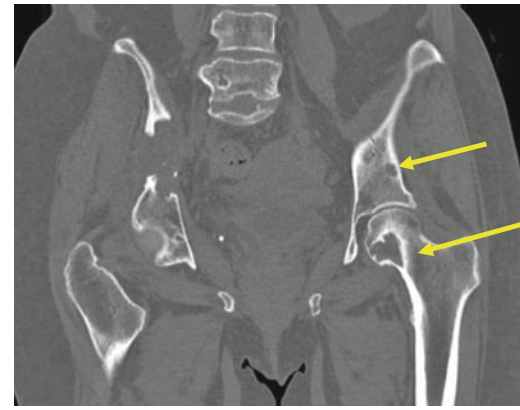

Fig. 7.3 Multiple lytic bone lesions involving the pelvis and femur from clear cell RCC. *Yellow arrow* shows sites of bone metastases from renal cancer

Approximately 20 % of patients present initially with paraneoplastic symptoms, while up to 40 % can develop some form of paraneoplastic symptoms during their disease course. After nephrectomy, the recurrence of a previous paraneoplastic syndrome should alert for possible disease progression. Because of its propensity for causing paraneoplastic symptoms, RCC has historically been called one of the "great masqueraders" of medicine [2, 3].

7.1.2.1 Hypercalcemia

This is the most common of the paraneoplastic syndromes, affecting 13–20 % of patients with RCC. Approximately 75 % of patients presenting with hypercalcemia have advanced disease, while about half have bone metastasis. Non-metastatic hypercalcemia is secondary to the elaboration of humoral peptides by RCC. These include PTHrP, IL-1, TNF, TGF, and OAF. The clinical picture can be very polymorphic. Symptoms can range from nonspecific symptoms such as asthenia, headache, lack of appetite, nausea, vomiting, constipation, polyuria, and polydipsia (due to nephrogenic diabetes insipidus) to specific clinical syndromes such as hypercalcemia or erythrocytosis or anemia. Hypercalcemia is a life-threatening condition that typically manifests with confusion, constipation, or nausea. Profound lethargy or even comatose condition has been noted. Physical findings include decreased deep tendon reflexes and an impaired level of consciousness. Patients may be dehydrated secondary to loss of renal concentrating ability and subsequent polyuria. Laboratory studies in affected patients reveal hypercalcemia, decreased levels of PTH, and 1,25-vitamin D and renal phosphate wasting. ECG findings include increased PR and QT intervals with eventual bradyarrhythmias and asystole. Treatment is mainly with repletion of volume with IV fluids and loop diuretics as needed. Bisphosphonates such as pamidronate or zoledronate are effective for long-term management. It has been suggested that the most effective way to treat the hypercalcemia is to treat the cause, with nephrectomy for localized disease and systemic therapy for metastatic RCC [2, 4].

7.1.2.2 Hypertension

Up to 40 % of patients with RCC develop hypertension as a paraneoplastic manifestation. Hypertension is typically associated with low-grade, clear cell tumors. Potential mechanisms include renin secretion, ureteral or parenchymal compression, presence of an arteriovenous fistula, and polycythemia. The sequence of events is believed to be as follows: local renal parenchymal compression and ureteral obstruction causes renin secretion, which then contributes to hypertension. Elevated serum renin levels have been found in 37 % of patients with RCC. Treatment for hypertension caused by RCC is nephrectomy; 85 % will become normotensive after such a procedure [2, 3].

7.1.2.3 Polycythemia

This is seen in 1–8 % of RCC patients, mainly mediated by erythropoietin (EPO), a glycoprotein produced by tumor cells and peritubular renal interstitial cells that promotes red blood cell production in the bone marrow. Elevated EPO levels have no prognostic significance. Patients with high EPO levels develop anemia more often than polycythemia [2, 3]. Interestingly, although two-thirds of patients with RCC have elevated EPO levels, only 8 % experience erythrocytosis.

7.1.2.4 Non-metastatic Hepatic Dysfunction (Stauffer's Syndrome)

In 1961, Stauffer noted hepatic laboratory abnormalities in a patient with RCC with no evidence of hepatic metastases. These resolved with nephrectomy but returned with disease recurrence. Incidence of this so-called Stauffer's syndrome is 3–20 %. Patients with this syndrome present with hepatosplenomegaly, fevers, and weight loss. It is characterized by transaminitis and abnormal hepatic synthetic function. In two-thirds of patients, nephrectomy led to resolution of Stauffer's syndrome. One-year survival was found to be 88 % in patients whose liver enzymes normalize after nephrectomy, compared to 26 % if they remain elevated [2, 3, 5, 6].

7.1.2.5 Constitutional Symptoms

One-third of RCC cases present with constitutional symptoms like fever, weight loss, and fatigue. Twenty to thirty percent can have fever, but only 2 % have it as a sole manifestation. In a study by Tsukamoto et al., 18 of 71 patients have elevated levels of IL-6, and 78 % of those with increased levels had fever [7]. In a study by Kim et al., cachexia, defined as hypoalbuminemia, weight loss, anorexia, or malaise, predicted worse survival after controlling for well-established indicators of prognosis including TNM stage, Fuhrman grade, and ECOG-PS [8].

7.1.2.6 Other Endocrine Abnormalities

Abnormal glucose metabolism has been described in RCC. There have been several case reports of either hyperglycemia or hypoglycemia. RCC tumors have been reported to have elevated intracellular levels of insulin, glucagon, and enteroglucagon when compared to controls.

RCC accounts for 2 % of all neoplasms that are responsible for Cushing's syndrome. This is secondary to enzymatic conversion of pro-opiomelanocortin to ACTH by the tumor. This ectopic ACTH drives cortisol secretion by the adrenal glands. Post-nephrectomy these patient are at risk for postoperative Addisonian crisis [2, 3]; thus, clinicians should be cognizant of this potential complication.

Finally, elevated serum beta-HCG levels can be found in 6 % of patients with RCC.

7.1.2.7 Non-endocrine Paraneoplastic Syndromes

Amyloidosis is seen in 3–8 % of patients with RCC. The amyloid protein found is AA. The mechanism hypothesized for AA deposition is prolonged stimulation of the immune system by either the malignancy or tumoral necrosis, leading to a rise in the levels of the acute phase reactant SAA. Initial patient complaints are weakness, weight loss, and syncope. Eventually the symptoms depend on which organ is involved.

Neuromyopathies are also described in RCC. They can be sensory or motor. Severity varies from nonspecific myalgias to a symptom complex reminiscent of amyotrophic lateral sclerosis.

7.2 Imaging (Table 7.3)

With the implementation of modern cross-sectional imaging modalities in clinical practice, the diagnosis, treatment, and surveillance of RCC have changed dramatically in the past two decades. As the incidental detection of small renal tumors has increased, this allowed earlier detection and treatment, consequently improving long-term survival rates [9, 10].

The major goals of imaging techniques are to correctly differentiate benign from malignant lesions and for early diagnosis, precise staging, and response evaluation to systemic therapy [11].

7.2.1 Ultrasound

Ultrasound (US) is often the first imaging technique used to evaluate patients with suspected RCC. Vascular flow detected by color Doppler US was reported to be strongly suggestive of conventional clear cell histology. Color Doppler US had a diagnostic accuracy similar to dynamic CT in most patients with renal solid tumors, and the color flow pattern was different among RCC

Table 7.3 Diagnostics of RCC: imaging modalities, their sensitivity and specificity

Imaging modality	Primary tumor		Perinephric extension		Lymph adenopathy		Venous thrombus/ tumor		Metastasis		Staging accuracy	IVC extension	
	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp		Sn	Sp
US	91	99	21		–	–	100	–	–	–	–	54	
CT	91	100	46	98	92	98	78	96	98	99	96	78	83
MRI	93	65	84	95	–	–	65	81			82	82	97
FDG-PET	60	100	–	–	75	100	–	–	63	100	94	–	–

subtypes. These observations suggest the use of color Doppler US as an additional tool in patients whose tumor is poorly attenuated or in those with contraindications for contrast medium and radiation [12]. When compared to CT scans, the accuracy of US to detect small renal tumors is low. The sensitivity for tumors that are <3 cm in diameter is only 67 % [13]. The deficiencies with conventional US are definitive identification of the following: complex cystic lesions, venous tumor thrombus extension, and verification of metastatic lesions. These shortcomings are due to the well-known inherent limitations of US imaging such as reliance on operator experience and on patient's constitution.

Contrast-enhanced US (CEUS) is a rapidly evolving technique using US-specific intravenous contrast agents in the form of microbubbles. A complete concordance between CEUS and CT in the differentiation of surgical and nonsurgical complex cysts was reported [14]. The sensitivity to detect tumor thrombus can reach 100 % if it involves the intrahepatic portion of the IVC, but it drops to 68 % if it lies below the level of the insertion of the hepatic veins. Depending on the patient's constitution, in 43.5 % of cases the IVC is not completely visualized [15]. It is the only available intraoperative imaging modality to ensure nephron-sparing surgery and to identify additional tumors. Under US guidance, minimally invasive procedures like biopsies and radiofrequency ablations can be performed [16].

Dynamic contrast-enhanced US can potentially be used in the era of antiangiogenic therapies to evaluate tumor response. An ongoing French national study will be able to define its utility in monitoring antiangiogenic therapy [17].

7.2.2 Computed Tomography (CT) Scanning

The gold standard for the diagnosis, staging, and surveillance of RCC is the CT scan [18, 19]. With multidetector-row CT (MDCT) scanners, one is able to obtain a true volume scan and ultrathin sections (<0.5 mm) with minimal time for motion artifact [20]. With the advent of triphasic (unenhanced, corticomedullary or arterial phase, and nephrographic phase) MDCT and 3D reconstruction, there is provision of accurate preoperative planning, especially for nephron-sparing surgery [21]. The degree of enhancement is a unique finding to differentiate conventional clear cell RCC from other subtypes and from angiomyolipoma [22]. Jinzaki et al. reported that clear cell RCC showed a peak attenuation value in the cortical nephrographic phase of >100 HU, whereas for other subtypes it is <100 HU [23]. The presence of homogeneous and prolonged enhancement significantly differentiates angiomyolipoma with minimal fat from RCC [24].

The staging accuracy with CT scans is 90 %. The detection of a normal adrenal gland in MDCT is associated with 100 % negative predictive value for metastasis [25]. For lymph node metastasis, the false-negative rate is 10 %, and false-positive rate ranges from 3 to 43 % [26, 27]. For M staging, there is an excellent agreement between MDCT and surgical pathology [27]. With the MDCT, tumor thrombus is accurately identified and localized.

Tumor response to antiangiogenic therapy can also be assessed with CT scanning. The application of RECIST criteria is limited in tumors with irregularity and diffuse invasion. So volumetric mean tumor attenuation in contrast-enhanced MDCT has been proposed as an alternative potential response criterion.

7.2.3 Magnetic Resonance Imaging (MRI)

MRI is the imaging modality of choice in patients with contrast allergy and functional renal impairment or who are pregnant. It is mainly used as a complementary problem-solving tool in selected cases of undefined renal lesions and suspected perinephric tumor spread or recurrence. The advantages of MRI include: absence of radiation, lack of need for standard iodinated contrast medium, and its high inherent contrast among different soft tissues [16]. Disadvantages are longer examination times, higher cost, and inferior capacity to detect lung metastasis. In patients

with renal insufficiency, the MRI contrast medium gadolinium has been associated with nephrogenic systemic fibrosis.

In a study by Pedrosa et al., the overall sensitivity and specificity of MRI to predict the histologic subtype were 92 and 83 % for clear cell and 80 and 94 % for papillary RCC, respectively [28]. MRI along with CT scans has difficulty in correctly identifying perinephric tumor invasion, distinguishing inflammation from tumor infiltration, and insensitivity in differentiating small collateral blood vessels from tumor extension in the lymphatics [29]. The sensitivity and specificity for detecting metastatic lymphadenopathy are low. It is highly sensitive and specific for detection of bone metastasis [30]. It is more sensitive than CT for detection of brain metastasis. MRI is a reliable method for evaluation of tumor thrombus. The accuracy is ranging from 65 to 100 % [16].

In regard to response evaluation to antiangiogenic therapy, it is still restricted to clinical trials because of poor standardization, methodologic challenges, limited sensitivity, and concerns related to potential harmful effects of MRI contrast agents.

7.2.4 FDG- PET

The increased background activity of healthy renal tissue and normal FDG excretion in urine can make visualization of primary renal cancers by PET difficult. 2-Deoxy-2-[18F]-fluoro-D-glucose (FDG) thus far has not offered any advantage over a standard imaging modality such as MDCT. In a retrospective review [31], the sensitivity and specificity of PET for detection of primary RCC were 60 % and 100 %, respectively, and with CT scan, these were 91.7 % and 100 %, respectively. It is also less sensitive than CT in the detection of metastasis to retroperitoneal lymph nodes and/or renal bed recurrence (75 vs. 92.6 %), lung metastases (75 vs. 91.1 %), and bony metastases (77.3 vs. 93.8 % of CT + bone scan). By using PET with an iodine-124-labelled antibody chimeric G250 (124I-cG250) against carbonic anhydrase-IX ("immuno-PET") for clear cell RCC, sensitivity was 94 % and specificity was 100 % [32]. Other markers under investigation are 18F-fluoromisonidazole (FMISO), a noninvasive tumor marker of tissue hypoxia, and 18F-fluorothymidine, a tracer that mirrors cellular proliferation.

FDG-PET/CT has the advantage to detect the metabolic activity of local recurrence that is not influenced by factors that jeopardize diagnosis of local recurrence with CT, such as migration of the adjacent normal organs into the renal fossa, postoperative scarring, and artifacts from surgical clips [33]. FDG-PET/CT can examine the whole body in one procedure without contrast agents. Park et al. demonstrated that, for the surveillance of high-risk RCC, FDG-PET/CT had results as good as conventional methods and were not influenced by the Fuhrman grade or the histological subtype. FDG-PET/CT is 89.5 % sensitive, 83.3 % specific, and 85.7 % accurate in detection of recurrence or metastasis.

7.3 Staging

Tumor stage, which reflects the anatomic spread and involvement by disease, is recognized as the most important prognostic factor for the clinical behavior and outcome of RCC. The first formal staging system proposed by Flocks and Kadesky in 1958 was based on the physical characteristics of the tumor and the location of tumor spread.

Currently the staging system that is followed is the tumor-node-metastasis (TNM) system. It was most recently revised in 2010 and is supported by both the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC). This is a dynamic staging method that changes continually on the basis of new evidence from clinical studies. It is based on data from large multicenter studies with a fairly good level of evidence.

The first TNM staging system was developed in 1978. Tumors are characterized on the basis of the degree of local extension of the tumor at the primary site (T), the involvement of regional lymph nodes (N), and the presence or absence of distant metastases (M). The classification may be clinical (cTNM) or histopathological (pTNM).

Table 7.4 Revised 2010 AJCC TNM staging system

Primary tumor (T)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
T1	Tumor 7 cm or less in greatest dimension, limited to the kidney		
T1a	Tumor 4 cm or less in greatest dimension, limited to the kidney		
T1b	Tumor more than 4 cm, but not more than 7 cm in greatest dimension and limited to the kidney		
T2	Tumor more than 7 cm in greatest dimension, limited to the kidney		
T2a	Tumor more than 7 cm but less than or equal to 10 cm in greatest dimension, limited to the kidney		
T2b	Tumor more than 10 cm, limited to the kidney		
T3	Tumor extends into major veins or perinephric tissues, but not into the ipsilateral adrenal gland and not beyond Gerota's fascia		
T3a	Tumor grossly extends into the renal vein or its segmental (muscle containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond Gerota's fascia		
T3b	Tumor grossly extends into the vena cava below the diaphragm		
T3c	Tumor grossly extends into the vena cava above the diaphragm or invades the wall of the vena cava		
T4	Tumor invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)		
Regional lymph nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in regional lymph node(s)		
Distant metastasis (M)			
M0	No distant metastasis		
MI	Distant metastasis		
Anatomic stage/prognostic groups			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1 or T2	N1	M0
	T3	N0 or N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

Regional lymph nodes for RCC are defined as the hilar, abdominal para-aortic, and paracaval nodes [34, 35]. Refer to Table 7.4 for a full description of the TNM staging system for RCC.

7.4 Clinical Prognostic/ Predictive Markers

Prognostic factors in RCC include:

- Anatomical (TNM classification, tumor size)
- Histological (Fuhrman grade, histologic subtype)
- Clinical (symptoms and performance status)
- Molecular features (described in Chap. 4)

All these factors are not accurate by themselves, but when combined, they improve accuracy of predicting outcome independent of treatment received. Hence, various prognostic models or nomograms have been proposed and designed. These models can be valuable tools for patient counseling, follow-up, clinical trial design, analysis, and interpretation [36].

7.4.1 Prognostic Factors in Non-metastatic RCC

Classical prognostic factors for non-metastatic disease include anatomical, histological, clinical, and molecular features.

Anatomic features are integrated in the TNM staging system. RCCs with higher T stage and lymph node and distant metastasis are associated with a worse prognosis and shorter survival [37, 38]. Involvement of the renal sinus fat appears to have worse prognosis [39, 40]. The current TNM staging does not distinguish between perirenal fat and renal sinus fat invasion, or between invasion of a muscular branch of the renal vein, and involvement of the entire renal vein (both staged as pT_{3a}). Involvement of ipsilateral adrenal gland confers dismal prognosis, and the outcomes are equivalent to stage IV disease [41]. Involvement of the IVC whether above or below the diaphragm is not prognostically different, but it has been shown that these patients have better prognosis when compared to patients with perinephric fat or nodal involvement [42].

Histological features include Fuhrman nuclear grade, histologic subtype, presence of sarcomatoid component, microvascular invasion, tumor necrosis, and collecting system inva-

sion. The most widely accepted histologic prognostic factor is Fuhrman nuclear grade developed in 1982 by Fuhrman et al. [43]. Four nuclear grades (1–4) were defined in order of increasing nuclear size, irregularity, and nucleolar prominence. Nuclear grade was more effective than each of the other parameters in predicting development of distant metastasis following nephrectomy. The value of Fuhrman grade in histological subtypes other than clear cell RCC has been disputed. The simplified version was as accurate as the classical four grades scheme when the grade was integrated into a prognostic nomogram [44].

Many studies have observed a significant association between histologic subtype and disease-specific survival in univariate analysis, with clear cell being the most aggressive tumor followed by papillary and chromophobe RCC. This prognostic value disappears in multivariable analysis suggesting that stage and grade have a higher impact on prognosis than the histology [45, 46]. RCC with sarcomatoid features have a dismal prognosis. Papillary tumors are divided into two groups with very different prognosis. Type I papillary tumors are low grade and multifocal and display a very favorable outcome, and type II are usually high grade and have an increased metastatic potential.

The presence of tumor necrosis is also a well-established independent indicator of poor prognosis for localized disease. Invasion of the collecting system is relatively rare but is associated with a worse prognosis, especially in lower stage disease.

Clinical prognostic features include performance status, local symptoms, cachexia, and anemia. The University of Michigan found that the mode of presentation (symptomatic vs. incidental) was an independent prognostic factor in the multivariate analysis for both disease-free and disease-specific survival [47]. Thrombocytosis is an independent prognostic marker, and it reflects a cascade of biological events correlated with tumor aggressiveness.

Several molecular and genetic tissue markers are investigated for prognostic significance. The prognostic role of von Hippel-Lindau (VHL) gene alterations and of hypoxia-induced factor 1alpha is controversial [48, 49]. VEGF is associated with more aggressive tumor phenotype. High carbonic anhydrase 9 (CA IX) levels have been associated with improved prognosis in advanced clear cell RCC [50]. Ki-67 has been found to be an independent prognostic factor in a multivariate analysis [50, 51], with high levels associated with poorer outcomes. Molecular markers have the potential to be used for screening, diagnosis, and follow-up, but at present have not been validated in well-designed multicenter prospective studies, hence limiting their clinical utility. Chapter 4 provides a more detailed description of molecular biomarkers in RCC.

A single prognostic feature does not yield sufficient predictive accuracy. Thus, investigators have combined different established parameters into algorithms or nomograms in order to improve prognostic accuracy. These tools are simple to use and are superior over standard multivariate regression models since they provide an estimate of the individual probability of outcome in a specific patient.

7.4.2 Prognostic Nomograms in Localized Disease

The first prognostic model was developed by Elson et al. in 1988, in 610 patients with recurrent or metastatic renal cell carcinoma to predict cancer-specific mortality. In 2001, investigators from Memorial Sloan-Kettering Cancer Center (MSKCC) introduced a postoperative nomogram for patients with localized RCC, which assigned points based on a combination of variables that included histology, tumor size, 1997 T stage, and symptoms at presentation. The aim was to predict the probability of RCC recurrence after nephrectomy in 601 patients. The predictive accuracy was 74 %, which however is no different from the TNM staging [52]. External validation was carried out in a European series and showed variable results [53]. The Kattan nomogram was updated by Sorbellini in 2005 [54]. These achieved 82 % accuracy in external validation but only in clear cell subtype (Fig. 7.4).

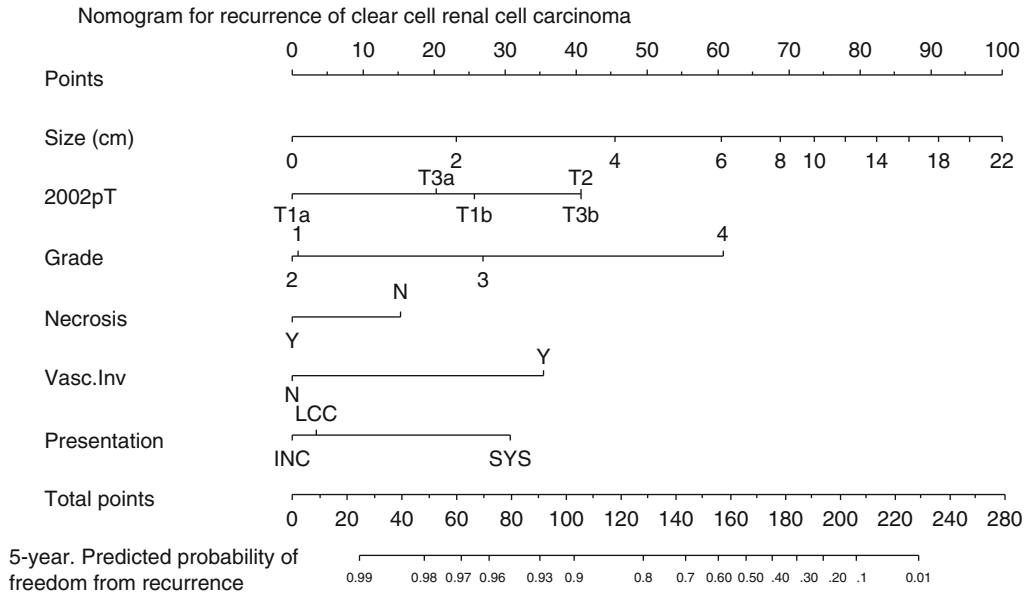

Fig. 7.4 Postoperative nomogram to predict recurrence in localized clear cell RCC (Reprinted from Sorbellini et al. [54], January 2005 with permission from Elsevier)

The Mayo Clinic introduced a prediction model to assess cancer-specific survival, in patients with clear cell RCC who underwent radical nephrectomy. In multivariable analysis TNM stage, tumor size, nuclear grade, and tumor necrosis are found to be significant. The predictive accuracy of the SSIGN was 81–88 % in external validation [55].

In 2003, Leibovich et al. developed an algorithm to predict progression to metastases after radical nephrectomy in clinically localized clear cell RCC. Tumor stage, size, grade, necrosis, and regional lymph node status were statistically significantly associated with progression to metastases. The metastases-free survival rates were 86.9 % at 1 year and 74.1 % at 5 years [56].

Another prognostic model has been the UCLA Integrated Staging System (UISS). The UISS was developed using the kidney cancer database from the University of California Los Angeles Kidney Cancer Program with the goal of providing a simple and accurate algorithm for predicting survival using variables that are available in any modern medical practice. In the initial study by Zisman et al. [57], patients were grouped based on TNM stage, Fuhrman grade, and ECOG performance status. This algorithm differed from the MSKCC nomogram, as it is limited to patients with clear cell histology and included other factors like nuclear grade and histologic tumor necrosis. The presence of symptoms at presentation, which was a prominent feature in the Kattan's nomogram, was not significant in this analysis after adjusting tumor stage, size, regional lymph node status, nuclear grade, and necrosis. In this study it was found that tumors measuring >10 cm were 48 % more likely to metastasize when compared to tumors <10 cm, after adjusting for other statistically significant pathologic features (see Fig. 7.5). The purpose was mainly to define subgroups with different risks of death following nephrectomy (Fig. 7.6).

In an international multicenter study by Patard et al., UISS was used to stratify both localized and metastatic RCC into three different risk groups. For localized disease, the 5-year survival rates were 92 %, 67 %, and 44 % for low-, intermediate-, and high-risk groups, respectively. A trend toward a higher risk of death was observed with increasing UISS risk category. This study confirmed the general applicability

UISS	1997 TNM Stage	Furman's grade	ECOG	2-years survival %	2-years survival SE	5-year survival %	5-year survival SE
I	I	I, 2	0	96	2.5	94	2.5
II	I	I, 2	1 or more	89	3.8	67	6.4
	I	3, 4	Any				
	II	Any	Any				
	III	Any	0				
	III	1	1 or more				
III	III	2-4	1 or more	66	6.5	39	2.8
	IV	I, 2	0				
IV	IV	3, 4	0	42	3.5	23	3.1
		1-3	1 or more				
V	IV	4	1 or more	9	6.2	0	4.0

Fig. 7.5 UISS categorization Table with 2- and 5-year projected survivorships (Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Zisman et al. [68])

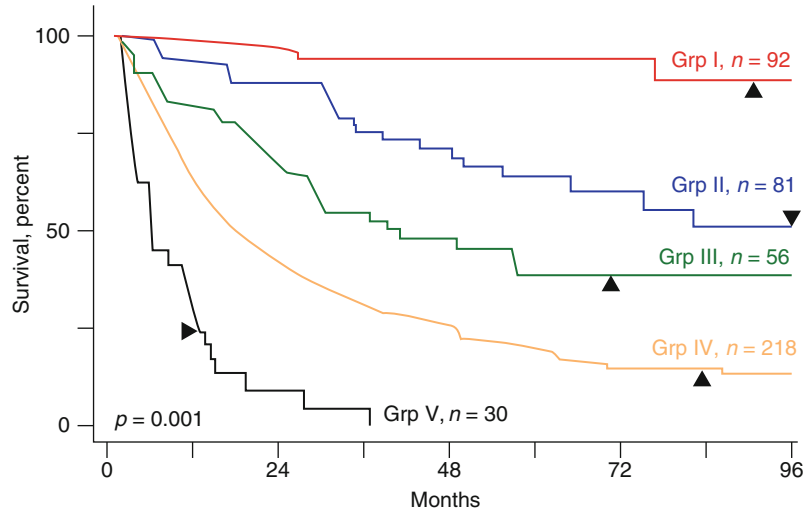

Fig. 7.6 Kaplan-Meier survival analysis of the study population according to the UISS categories. *Black triangles* mark the ten patients at risk point (Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Zisman et al. [68])

and accuracy of the UISS for predicting survival in localized RCC. The predictive accuracy was 86 % at 2 years, which is significantly superior to that of the TNM system alone. The high predictive accuracy combined with its validity and robustness across different populations made it a reliable and useful tool for clinical practice [58].

In 2007, Karakiewicz et al. [59] proposed a nomogram for prediction of RCC-specific survival. This is similar to the UISS, but tumor size is used as a continuous variable and the ECOG performance status is replaced by symptoms that distinguish asymptomatic and local and systemic symptoms. The predictive accuracy at 10 years was 89 % in the external cohort validation and had the highest predictive accuracy.

7.4.3 Prognostic Factors in Metastatic Disease

In metastatic setting, the prognostic impact of the primary tumor characteristics disappears. The classic anatomic factors (stage, size, perinephric fat, venous or adrenal invasion) have very limited prognostic role. The location, multiplicity, and resectability of the *metastasis* play a significant role in prognosis. Presence of multiple lung and brain metastasis and involvement of bone especially spinal location indicate worse prognosis. Presence of sarcomatoid differentiation is associated with very poor prognosis. However, the most important *clinical prognosticator* appears to be performance status.

Biological prognostic factors include low hemoglobin, elevated lactate dehydrogenase, and high corrected serum calcium and inflammatory markers. Several of these pretreatment clinical features have been associated with shorter survival, and thus identification of these prognostic factors has led to the development of risk stratification models.

In the metastatic setting, the combination of several variables has higher predictive accuracy than independent variables. The two most adopted are classification systems of the *French group of immunotherapy* and the *MSKCC model(s)*.

The Groupe Franc¸ais d'immunotherapie enrolled 782 mRCC patients over a 6-year period. This group developed and validated a prognostic model based on performance status, number and location of metastases, interval between diagnosis and systemic treatment, hemoglobin level, neutrophil count, and other biological signs of inflammation. This was designed to predict progression and survival following cytokine-based immunotherapy and stratified patients according to the number of adverse prognostic factors into three prognostic groups—good, intermediate, and poor risk—with median survival rates of 42, 15, and 6 months, respectively. The four independent factors predictive of rapid progression under treatment were: presence of hepatic metastases, short interval from renal tumor to metastases (<1 year), more than one metastatic site, and elevated neutrophil counts. Patients with at least three of these factors have over 80 % probability of rapid progression despite treatment [60].

The MSKCC model was developed by Motzer et al. and used data from patients with RCC who received treatment with IFN-alpha. The database was a retrospective study of 670 advanced renal cancer patients treated in successive clinical trials at MSKCC to define pretreatment features predictive of survival. The five risk factors associated with shorter survival were low Karnofsky performance status (<80 %), high lactate dehydrogenase (>1.5 times upper limit of normal), low serum hemoglobin (< lower limit of normal), high corrected serum calcium (>10 mg/dL), and interval from diagnosis to treatment of less than 1 year. Three-year survival for the favorable-risk (0), intermediate-risk (1–2), and poor-risk (>/=3) groups were 31 %, 7 %, and 0 %, respectively. The median survival rates in the three risk groups were 20, 10 and 4 months [61], respectively.

The MSKCC criteria were validated and additionally elaborated by an independent group at the Cleveland Clinic in a cohort of 308 untreated mRCC patients. In addition to the MSKCC criteria, prior radiotherapy and the presence of more than one site of metastases also had negative prognostic value [62].

MSKCC investigators then developed another prognostic model for patients who have failed cytokine therapy. Factors associated with shorter survival were low Karnofsky performance status, low hemoglobin level, and high corrected serum calcium. The median survival times with 0, 1,>/=2 risk factors were 22, 11.9, and 5.4 months, respectively [63].

These prognostic risk profiles are derived from the era of immunotherapy, and it is unclear if these prognostic factors are relevant to contemporary patients predominantly treated with VEGF-targeted therapy. It was essential to validate these prognostic models in the era of targeted therapy. In the study by Motzer et al., treatment-naïve mRCC patients were randomized to either sunitinib or INF. The predefined MSKCC risk factors predicted longer PFS with sunitinib [64]. In a recent multicenter, retrospective study led by Heng et al. in metastatic RCC patients, treated with VEGF-targeted therapies, four out of the five MSKCC adverse prognostic factors (anemia, hypercalcemia, poor performance status, shorter time from diagnosis to initiation of therapy) were identified as independent determinants of OS outcome. Additionally, presence of bone metastases, neutrophilic leukocytosis, and thrombocytosis were noted to be independent adverse prognostic factors. Patients were segregated into three prognostic groups depending on these six factors. Two-year survival rates for the favorable-risk (0), intermediate-risk (1–2), and poor-risk (3–6) groups were 75 %, 53 %, and 7 %, respectively (Fig. 7.7). This study established a contemporary prognostic model that is clinically applicable to determine OS outcomes in the targeted therapy era [65].

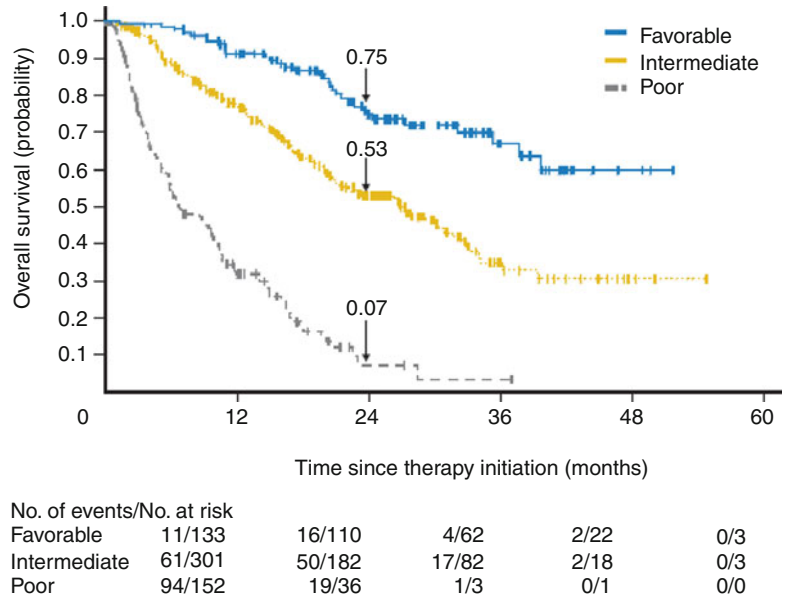

Fig. 7.7 Overall survival probability according to time after therapy initiation and risk group (Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Heng et al. [65])

Majority of the targeted therapies in mRCC were approved based on PFS benefit; however, it was not clear if PFS is an adequate surrogate of OS in advanced RCC. In a retrospective study evaluating 1158 RCC patients who received targeted therapy, median OS for patients who progressed at 3 months was 7.8 months, compared with 23.6 months for patients who did not progress at the 3-month time point ($P<0.0001$). Similarly, using a 6-month cutoff instead of 3 months, progressing patients had a median OS of 8.6 months compared with 26 months for patients who did not progress ($P<0.0001$). This study concluded that patients with advanced RCC who progressed on contemporary targeted therapy had an approximately three times increased risk of death compared to patients who are progression free at the same time point. This study suggested that PFS may be a meaningful intermediate endpoint for OS in patients with mRCC who receive treatment with novel agents [66].

SWOG 8949 prospectively evaluated the role of debulking nephrectomy in advanced RCC. Patients on the nephrectomy arm continued to have survival benefit at 9 years of follow-up, with risk reduction by 26 %. This benefit was seen across all predefined strata, including performance status and the presence or absence of lung metastasis and measurable disease. The role of cytoreductive nephrectomy (CN) in this new era of VEGF-targeted therapy was retrospectively evaluated by Choueiri et al. After adjusting for established prognostic risk factors, CN reduced the risk of death by 32 % (95 % CI: 0.46–0.99, $P=0.04$). In the subgroup analysis, marginal survival benefit is seen in patients in the poor-risk group ($p=0.06$) and Karnofsky performance status <80 % ($p=0.08$) [67].

Case Vignette

A 58-year-old man presented with flank pain and left hip discomfort. He has no other medical problems, never smoked, and only takes a statin for hyperlipidemia. His urinalysis showed microscopic hematuria. A complete blood count showed anemia with a hemoglobin concentration of 10 g/dL. His kidney and liver function tests were normal. Computed tomography (CT) scans revealed a large 12 cm solid right renal mass. Regional lymph nodes were not enlarged (Fig. 7.8a). A plain radiograph of the pelvis demonstrated a sclerotic lesion in the left femoral head (Fig. 7.8b). There was no other

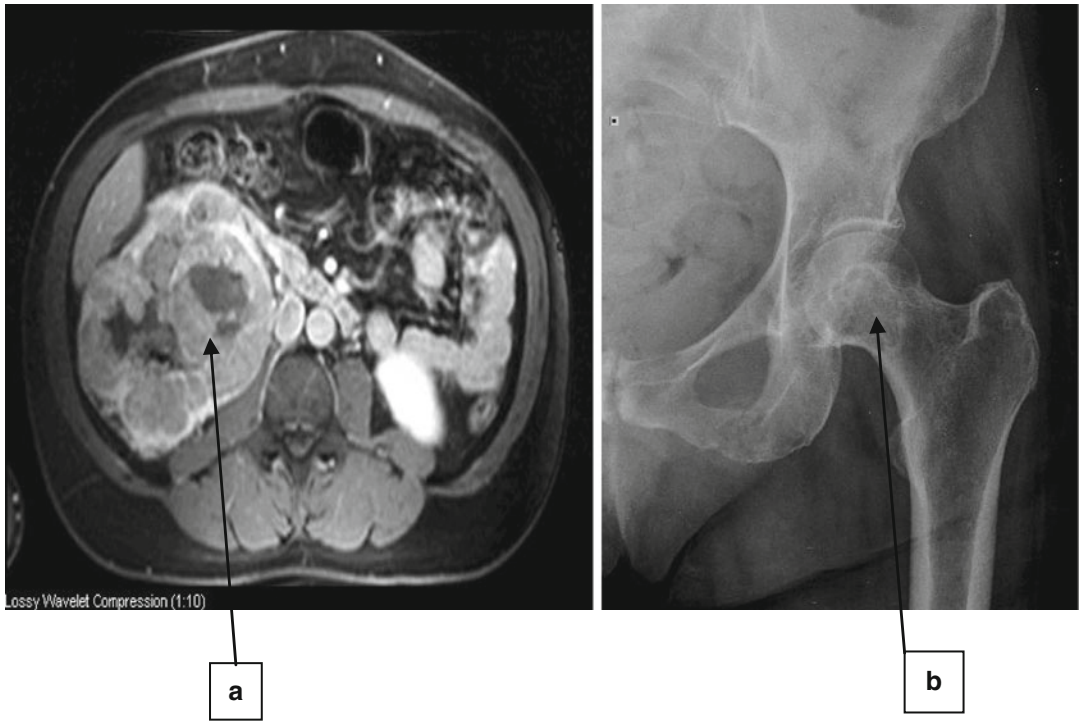

Fig. 7.8 (**a**) Right kidney with a primary renal mass. (**b**) Lytic bone lesion in the femoral head

evidence for metastatic disease in the rest of the CT images. Bone scan showed only uptake in the left femoral head. This patient underwent a right radical nephrectomy and was found to have clear cell RCC; no nodes were involved. Biopsy of the femoral head mass was positive for metastatic clear cell cancer. An MRI of the left hip suggested that surgical resection was feasible. Because of the oligometastatic nature of this patient's disease, he was considered for surgical metastasectomy, subsequently undergoing an R0 resection of the femoral head mass with placement of an artificial hip. He also received postoperative radiation therapy to the left hip. He was started on bisphosphonate therapy. Final pathologic stage was T2N0M1. He remains metastasis-free 2 years after his last operation. He is ambulating normally. Systemic therapy is planned only at the time of tumor recurrence.

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Hereditary Kidney Cancer Syndromes

8

Shu Pan and Brian Shuch

Contents

Key Points

- Approximately 5–8 % of kidney cancer cases may have a hereditary component, and the percentage is likely to increase with improved diagnostics.
- Patients with an atypical clinical presentation, such as concomitant involvement of other organ systems, bilateral tumors, early age of onset, or significant family history, should be screened for a genetic basis of kidney cancer (see Table 8.1).
- Aggressive surgical excision of all renal tissue is no longer the standard of care. Enucleation and partial nephrectomy have been used with success to preserve renal function in many renal cancer syndromes.
- Close observation with a 3 cm threshold for surgical intervention can be the guiding principle for managing von Hippel-Lindau (VHL), hereditary papillary renal cell (HPRC), and Birt-Hogg-Dubé (BHD).
- Aggressive hereditary kidney cancers such as SDH and HLRCC should not be observed; these patients should be managed with early surgical intervention with wide margin.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_8

Table 8.1 Features that should raise suspicion for a hereditary renal cancer syndrome

Features suggestive of hereditary RCC	
Family history of RCC or confirmed syndrome	
Bilaterality, multifocality, early age of onset	
Histology: non-clear cell with unusual features, e.g., papillary type II morphology with orangophillic nucleoli, or hybrid oncocytic neoplasms	
Associated tissue manifestations	Dermatologic: skin leiomyoma, folliculofibroma, along with multiple other skin features in TSC, BHD, and Cowden's disease
	Ophthalmologic: astrocytic hamartoma (phakoma), retinal angiomas
	Neurologic: hemangioblastoma, giant cell astrocytoma, cortical tubers, and subependymal nodules
	Endocrine: pheochromocytoma, paraganglioma, adrenal nodules, pancreatic cysts, and neuroendocrine tumors
	Gynecologic: early onset of uterine fibroids (<30)
	Pulmonary: lung cysts

8.1 Introduction

In 2014, there are over 65,000 newly diagnosed cases of kidney cancer, responsible for nearly 14,000 deaths [1]. While the death rates fell by 0.9 % per year from 2006 to 2010 [1], it is still very much a lethal disease. Current expert opinion suggests that while the overwhelming majority of RCC is sporadic in nature, hereditary kidney cancer accounts for 5–8 % of cases [2–4]. This number may be a gross estimation and with the explosion of next-generation sequencing techniques. More syndromes are expected to be discovered, some with highly complex inheritance patterns.

Currently, there are more than a dozen confirmed types of hereditary kidney cancer syndromes. Many share overlapping dysregulation in metabolic pathways involved in the cells ability to respond to changes in nutrients, oxygen sensing, and iron metabolism [5]. While there are shared dysregulation in similar pathways, their degree of renal penetrance and aggressiveness vary in addition to the types of extrarenal manifestations. The more prominent hereditary RCCs will be discussed in the following chapter, along with a summary of their management.

8.2 Clinical Recognition

Management of hereditary RCC may have substantial differences from that of its sporadic counterpart, and therefore, recognition is critical. While clinicians should avoid screening every patient with a renal mass for genetic anomalies, a certain pattern of clinical presentation and family history should trigger further workup (Table 8.1). First and foremost, a positive family history of diagnosed syndrome or bilateral/multifocal renal lesions should prompt further investigation, even if the patient in question is noted to have benign neoplasm of the kidney. Age of onset of ≤46 years of age is another criterion, derived from a study based on the SEER database along with the National Cancer Institute (NCI) experience. It was found that 70 % of hereditary kidney cancer fell below the bottom decile in age, and referral to genetic counselors based on this threshold could maximize sensitivity and specificity for detection of a hereditary form of kidney cancer [4]. Additional features foretelling of hereditary RCC include non-clear cell with unusual histologic features [6] and associated physical manifestations (i.e., dermatologic, gastrointestinal, ophthalmologic, neurologic, endocrine, gynecologic, and pulmonary). As such, Reaume and colleagues published a clinical practice guideline aimed to guide clinicians in identifying patients with hereditary RCC [6].

8.3 Syndrome Manifestations

8.3.1 Von Hippel-Lindau

Von Hippel-Lindau (VHL) disease was the first heritable RCC to be discovered. Its characterization in the early twentieth century led to

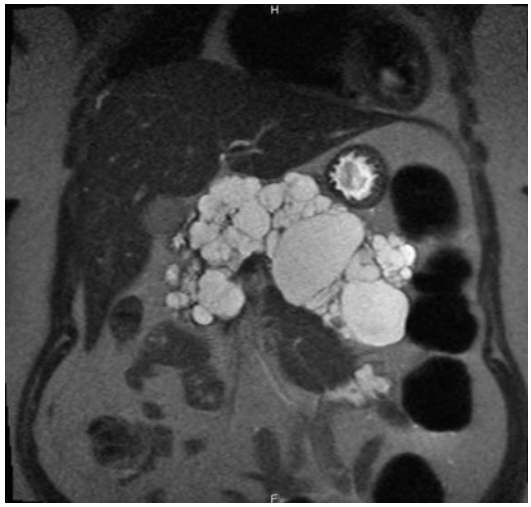

Fig. 8.1 Coronal T2-weighted MRI image of a VHL patient in whom the pancreas was completely replaced with cysts

subsequent breakthroughs applicable to even the sporadic form of the disease, where over 90 % of cases are found to harbor the similar genetic alterations [7]. Determination of the responsible gene by the NCI began with the observation that there was a consistent loss of chromosome 3p in affected individuals [8], but it was not until sometime later that the researchers localized the locus to 3p25.1 [9]. With an autosomal dominant pattern of inheritance, *VHL* has a high degree of penetrance. The *VHL* gene functions as a classic tumor suppressor [10] and requires a "second hit" for loss of the wild-type allele. The *VHL* gene encodes for a protein, VHL, that modulates the activities of hypoxic-induced factor (HIF), most notably HIF-1a and HIF-2a. When VHL is either absent or nonfunctional, the HIFs are stabilized even under normoxic conditions, resulting in unchecked shift toward a pseudo-hypoxic state with upregulation of anaerobic metabolism, angiogenesis, and carcinogenesis.

The clinical manifestations of VHL include hemangioblastomas (of the retina, brain, and spinal cord), pheochromocytomas, pancreatic cysts or neuroendocrine tumors (Fig. 8.1), cystadenomas of the epididymis or broad ligament, and bilateral, multifocal kidney cysts or tumors [11]. While renal cysts in non-syndromic patients are predominantly benign, individuals with VHL tend to have cysts lined with malignant cells, and there may be other microscopic foci of RCC [12]. An estimated 70 % of affected individuals develop RCC by the age of 60. Not only do these people present with multiple lesions, their age of onset was also noted to be an estimated 25 years earlier than sporadic RCC [13].

VHL has also been divided into several subtypes based on the presence of pheochromocytoma, RCC, and type of genetic mutation. Type 1 VHL typically presents with RCC but low risk for pheochromocytomas, and its genetic alterations are germline deletions or truncating mutations. Type 2 VHL carries a low risk for RCC but the presence of pheochromocytoma is a characteristic; this type or subtype of VHL has underlying missense mutation of the affected gene [14, 15]. While useful to help understand which patients have the highest risk of each condition, clinically overlap has been observed.

8.3.2 Hereditary Papillary Renal Cancer

Early in the 1990s, Zbar et al. discovered multiple families affected by papillary renal cell carcinoma (pRCC) with a very high penetrance [16, 17]. Interestingly, the affected individuals displayed no loss of chromosome 3p, which lead the researchers to suspect a separate heritable RCC syndrome, hereditary papillary RCC (HPRC). This second hereditary RCC syndrome was later linked to 7q31 and found to be caused by a genetic mutation in the *MET* proto-oncogene [18]. *MET* encodes for a crucial tyrosine kinase receptor binding the hepatocyte growth factor (HGF), and the mutant variant demonstrates a high degree of phosphorylation corresponding to elevated enzymatic activity [19]. Activated MET signaling is believed insufficient in tumorigenesis alone, but nonrandom duplication of copies of the affected gene is commonly detected in HPRC perhaps contributing to carcinogenesis [20].

Unlike VHL, HPRC presents only with kidney tumors without any extrarenal manifestations. Without a set of distinguishing features, diagnosis

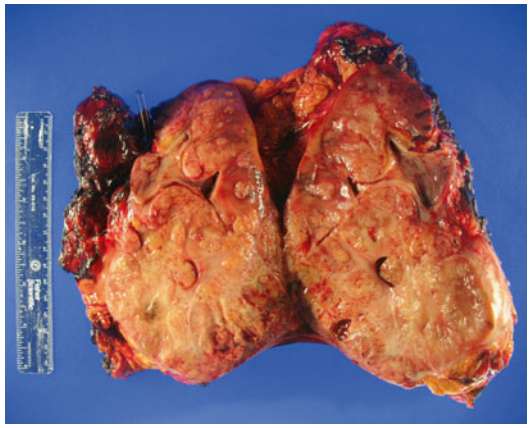

Fig. 8.2 Radical nephrectomy specimen of a 15 cm left renal mass. The tumor was believed localized to the lower pole of the kidney, but on final pathology, the tumor infiltrated throughout in all regions of the kidney

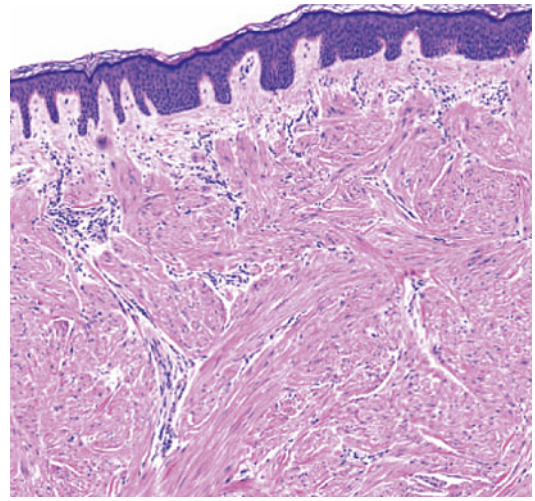

Fig. 8.3 5× magnification of a cutaneous shave biopsy of a cutaneous leiomyoma in a patient with HLRCC. The underlying dermis contains interlacing smooth muscle bundles and fascicles

can be difficult and may rely on a meticulous documentation of familial pedigree. The penetrance for this disorder is considerable. Moreover, the risk of RCC only increases with age, upward to 67 % by age 60 [21, 22]. Generally speaking, the disease is detected after the age of 30, but subtypes with earlier onset have been described [23]. HPRC presents with papillary type 1 [24], and it is not uncommon for there to be several thousand small papillary foci to be present in the renal parenchyma of affected individuals [25].

8.3.3 Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC)

Reed syndrome was first described in 1958 by dermatologists who treated a group of patients with distinct cutaneous leiomyomas [26]. It was not until sometime later that clinicians discovered that many patients had an inherited form of both cutaneous and visceral leiomyomatosis as well as an aggressive form of RCC (Fig. 8.2), leading to the syndrome being renamed hereditary leiomyomatosis and renal cell carcinoma (HLRCC) [27–29]. In this autosomal dominant disease, genetic linkage studies localized the affected gene to 1p42.3-43 that encodes for *fumarate hydratase* (*FH*) [30, 31]. This enzyme is an integral part of the Krebs cycle, catalyzing a hydration reaction of fumarate to malate. HLRCC renal tumors demonstrate a loss of heterozygosity of the wild-type *FH* allele, shifting cellular metabolism toward aerobic glycolysis (the Warburg effect) [32, 33].

HLRCC presents with a unique constellation of signs and symptoms: leiomyoma (80 %) (Fig. 8.3), early-onset uterine fibroids in women (90 %), aggressive RCC (20 %), and, less frequently, macronodular adrenal hyperplasia (8 %) [34–36]. Initial descriptions of the associated RCC were papillary type 2, but HLRCC can present with a variety of different morphologic features. A common theme in their pathologic appearance includes eosinophilic nucleoli and perinuclear halos [33]. The International Society of Urologic Pathology now considers HLRCC renal tumors to be a distinct subtype [37].

8.3.4 Birt-Hogg-Dube (BHD)

BHD was discovered by a group of Canadian dermatologists in 1977. Their study of an extended family revealed 15 out of 37 members after the age of 25 developing fibrofolliculomas and associated trichodiscomas or acrochordon

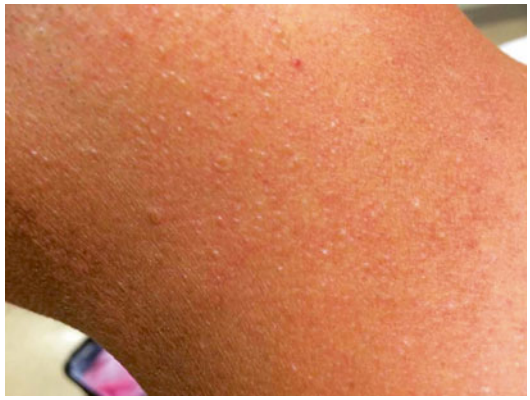

Fig. 8.4 Image of left neck of an individual with BHD demonstrating multiple small fibrofolliculomas

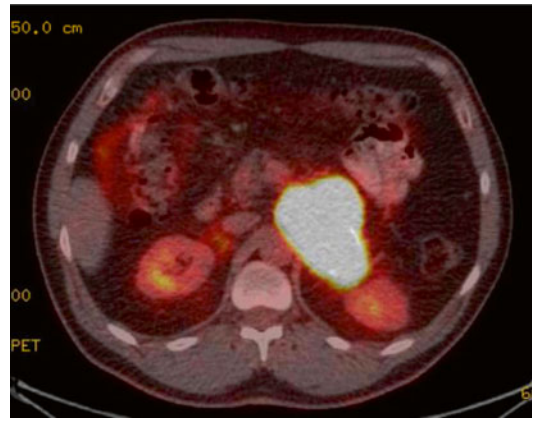

Fig. 8.5 A PET/CT imaging from a 9 cm left paraganglioma above the kidney and adjacent to the superior mesenteric artery. The patient had anxiety and intermittent mood swings due to massively elevated catecholamines. The family history was significant for a GI stromal tumor, and he was found with an *SDHB* alteration

(Fig. 8.4) [38]. The fibrofolliculomas were extension of epithelial strands beyond the infundibulum of the hair follicles. The incidence of BHD was noted to be 1 in 200,000 and transmitted in an autosomal dominant fashion. Further linkage analysis confirmed the gene location to 17p11.2 [39], which encodes for *folliculin* (*FLCN*). The gene in question behaves like a classic tumor suppressor and was found to participate in a complex that regulates downstream functions of AMPK and mTOR [40, 41]. Hasumi and colleagues determined loss of FLCN-upregulated activities of mTORC1 and mTORC2 [42].

In addition to the skin manifestations that are pathognomonic to BHD, the affected individuals also have a greater than 50-fold increase risk in lifetime pneumothoraces, a likely sequela of their propensity in developing lung cysts [43]. After adjusting for age, those with BHD were about seven times more likely to develop renal tumors [43]. With an average onset of age 50, renal lesions in the setting of BHD tend to be bilateral and multifocal in their occurrence [44–46]. The most common tumor in BHD is a hybrid oncocytic neoplasm, which contains both chromophobes with oncocytic features [47]. These tumors tend to have better prognosis due to their relatively indolent progression: less than 5 % of affected individuals go on to develop metastatic disease [48]. Clear cell and papillary histologic subtypes are found in less than 10 % of tumors, but when present, they portend a significantly worse prognosis.

8.3.5 Succinate Dehydrogenase B/C/D

Hereditary paraganglioma and pheochromocytoma syndromes were a group of syndromes closely associated with the development of chromaffin cell tumors, namely, pheochromocytomas and, their extra-adrenal counterparts, paragangliomas (Fig. 8.5). It was initially postulated that only 10 % of such tumors possessed a genetic component, but recent gene studies have confirmed that as high as 35 % of these neoplasms without a definable syndrome are caused by a hereditary predisposition [49]. Since 2000, it became recognized that many of these syndromes were related to an alteration in a common enzyme succinate dehydrogenase; hence, the syndromes are considered to have succinate dehydrogenase deficiency (SDH). There are multiple constituents to the SDH enzyme complex that function together as an important enzyme in the Krebs cycle, converting succinate to fumarate. Located in the inner mitochondrial membrane adjacent to the matrix, SDH not only participates in the Krebs cycle but also as complex II in the electron transport chain. Its dysregulation directly results in hypoxic metabolism and aggressive neoplastic growth consistent with the Warburg effect [50].

Pertaining to hereditary kidney cancers, however, alterations in only SDH subunits B/C/D have been implicated. This association was first characterized in 2004, and it was later found that 5 % of familial RCC without a diagnosed syndrome could be mapped to *SDHB* germline mutation [51, 52]. A germline mutation in genes coding for the other subunits SDHC and SDHD has also been linked to an increased risk of RCC. Similar to HLRCC, kidney tumors in the setting of SDH tend to follow an aggressive course. In addition to chromaffin and kidney tumors, recent findings suggest that SDH mutations may also increase the risk of gastrointestinal stromal tumors [53].

8.3.6 Tuberous Sclerosis 1 and 2 (TSC1, TSC2)

The tuberous sclerosis complex is an autosomal dominant condition well known to the dermatologic and neurologic communities. Its underlying genetic mutations were successfully linked to *TSC1* (9q34), *hamartin*, and *TSC2* (16p13), *tuberin* [54, 55]. Similar in its signaling pathway to VHL, the loss of TSC2 in animal models has shown to upregulate HIF and mTORC1 [56, 57]. While hamartin and tuberin represent the most recognized germline mutations, the genetic basis for the renal pathologies in the setting of TSC remains ill defined.

The hallmark of tuberous sclerosis is its dermatologic manifestations. Nearly all of the affected individuals demonstrate some form of skin lesions: ash-leaf spots (hypopigmented macules), angiofibromas (also known as fibroadenomas) (Fig. 8.6), and shagreen patches found most commonly on the lower trunk. A significant portion of these people also have intracranial lesions, with the most well-known lesions being the glioneuronal hamartomas, or tubers. Moreover, they may also harbor subependymal giant cell astrocytomas (SEGAs). Surprisingly, the degree of cognitive dysfunction and epileptic risk are only loosely correlated with the burden of brain lesions.

Renal manifestations of TSC tend to be highly penetrant. The common lesions include angiomyolipomas, renal cysts, and less commonly RCC, which can be of any subtype [58]. Despite the benign nature of AMLs, mass effects are common, and the clinician should always be wary of the risk of hemorrhage for lesions greater than 4 cm. It is worthy of noting that some patients with TSC suffer from chronic kidney disease, which may be the result of renin-dependent hypertension or direct parenchymal compression. While RCC arise in <5 % of patients with TSC, the lesions tend to have an early age of onset and a more aggressive clinical course [59].

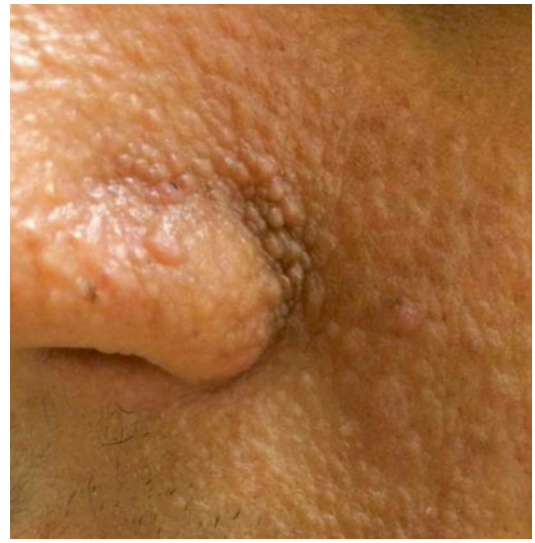

Fig. 8.6 Image of the nasal labial fold of a young man with TSC showing extensive angiofibromas/fibroadenomas

8.3.7 Cowden's Disease/PTEN

Cowden's disease is an autosomal dominant disease thought to affect 1 in 200,000 persons, an estimate now questioned to be an underestimation [60]. The gene in question was localized to 10q22, also known as *PTEN*, which serves as a tumor suppressor [61, 62]. Cowden's disease (CD) presents with a host of cutaneous and mucocutaneous lesions. The hallmarks of CD are the benign hair follicle tumors termed trichilemmomas [63]. CNS pathologies are also common, where the affected individuals manifest macrocephaly and cerebellar

white matter disorganization (Lhermitte-Duclos disease). They also demonstrate symptoms of ataxia, tremors, and cognitive dysfunction. From an oncologic standpoint, CD patients tend to develop epithelial neoplasms, including malignancies of the breast, uterus, thyroid, colon, and prostate. With regard to renal neoplasms, individuals with CD demonstrate 4 % incidence of developing RCC, which is approximately 30× the lifetime risk of normal individuals [64]. In a series of patients with CD, 16 % had a history of kidney cancer involving all major histologic subtypes [65]. As such, patients with confirmed CD may benefit from early screening of kidney cancers.

8.3.8 Other Syndromes

There are a handful of lesser-known heritable syndromes with renal neoplasms still worthy of mention. First and foremost, Cohen and colleagues reported a family with hereditary kidney cancer similar to VHL notable for a balanced translocation of chromosome 3. The affected did not possess the classical extrarenal manifestations of VHL and tended to develop RCC later in life. In the ensuring decades, further population studies were conducted, namely, one involving the Danish cytogenetic and cancer registry [66]. This phenomenon is believed explained by a three-hit model of carcinogenesis: first an individual is born with an abnormal karyotype involving translocation of 3p, second there is a loss of the 3p fusion chromosome, and finally the remaining VHL allele undergoes a somatic mutation [67, 68].

Oncocytomas are the most commonly resected benign tumors of the kidney. While oncocytomas lack the invasive potential RCC, they can nevertheless cause symptoms secondary to mass effect. Approximately 10 % of oncocytomas are bilateral [69–71], and patients with bilateral lesions should be screened for BHD. In the cases of bilateral oncocytoma with negative screening for BHD, a diagnosis of bilateral multifocal oncocytomas is rendered. By the same token, a familial form of bilateral and multifocal oncocytomas has been coined familial renal oncocytoma (FRO) [72]. Patients with this condition should be observed closely, and prior to additional intervention, there should be consideration of renal biopsy. Lastly, there exists a variant form termed renal oncocytosis, where diffuse oncocytic nodules arise in the renal parenchyma, and many patients progress to renal failure [73]. The goal of management for these patients is centered on the maintenance of renal function through conservative measures.

8.4 Management

8.4.1 General Considerations

The management of hereditary renal carcinomas, though far from perfect, is not a terribly complex algorithm. Traditionally, bilateral nephrectomy was considered for patients with hereditary RCC. Such an approach predestined all of these patients to a lifelong dependence on dialysis. Some of the more fortunate individuals, who are free of recurrence, became candidates for renal transplantation [74, 75]. Current practice has departed from the paradigm of removing all renal tissue; instead, preservation of nephrons has become a principal objective. For VHL, BHD, HPRC, and familial renal cancer of unknown cancer (FRC), the 3 cm threshold for surgical intervention has been implemented with success. Utilizing systematic resection of tumors in a stepwise fashion, from most accessible to the most challenging, the surgeon and anesthesiologist maximize hemodynamic stability for the longest duration possible. Enucleation, taking no additional margin of benign tissue, has also become the preferred technique in several forms of hereditary RCC. It has been utilized successfully with good outcome in VHL, BHD, and even HPRC [76, 77]. On the other hand, patients with the more aggressive subtypes of hereditary RCC (HLRCC and SDH) demonstrate greater propensity locoregional dissemination and thereby would benefit from a more aggressive surgical approach.

Complementary to surgical intervention, there are systemic therapies currently undergoing

clinical testing. These drugs are derived from the signaling pathways unique to the hereditary RCCs, but their application may someday encompass the sporadic forms of kidney cancer.

8.4.1.1 VHL/BHD/HPRC

Classically, a significant majority of patients with VHL would expire from metastatic kidney cancer. Traditional management to avoid dissemination of RCC was bilateral radical nephrectomies followed by renal replacement therapy [78]. Current opinion has shifted away from such a practice. Management of these complex patients now consists of early detection, meticulous surveillance (beginning in childhood), and a balanced consideration of surgical intervention and preservation of renal function. Nephron-sparing interventions such as partial nephrectomies are now first line, with the goal of limiting procedures, maximizing kidney function, and preventing risk of metastasis. Ablative techniques have also been utilized for patients who may be too ill for open or laparoscopic surgeries. BHD and HPRC can be managed in a similar fashion, as shown by a 10-year operative experience on hereditary kidney cancer published by Herring et al. [79].

8.4.1.2 HLRCC/SDH

The management of HLRCC differs from the other forms of inherited RCC. Unlike VHL or BHD, the 3 cm criterion for invoking surgical intervention does not apply—early resection with a wide margin is vital due to the infiltrative nature of these tumors (Fig. 8.2). HLRCC exhibits a very aggressive course, with early series reporting over one half of the patient population demonstrating regional or metastatic disease despite small primary tumors [34]. Although there is no consensus on a surveillance protocol, experts have proposed early genetic testing, screening, and abdominal imaging, followed by annual MRI due to the high lethality of the kidney cancer [35]. Akin to HLRCC, management of kidney tumors in SDH individuals involves early detection and wide surgical excision [80]. There has also been a recent report of VEGF-targeted therapy for a female patient with metastatic disease, where treatment with sunitinib was met with a near complete response [81].

Conclusion

Hereditary kidney cancer accounts for 5–8 % of the total number of RCC diagnosed, but its clinical significance cannot be overstated. Clinicians should have a heightened index of suspicion when a patient presents with bilateral, multifocal lesions at a young age and has a strong family history of RCC, or if he/she shows the characteristic extrarenal manifestations. Management of such patients follows three principal tenets: early diagnosis, rigorous surveillance, and interventions that maximize renal function. Genetic testing and management require a multidisciplinary team including a clinical geneticist. Ascertainment of the genetic defect not only dictates the course of treatment for the patient but also has bearing on the health management of the living relatives. Finally, surgical resection should be undertaken with the considerations of sound oncologic extirpation weighed against the preservation of renal function. Despite our advancements in genetics, surgical techniques, and pharmacotherapies, it is apparent that much work remains to fully delineate this group of heritable cancers.

Clinical Vignette

The patient is a 71-year-old female who had flank pain and underwent a laparoscopic, radical left nephrectomy, with initial pathology revealing high-grade T2bNxM0 RCC resembling possible collecting duct RCC. She was found to have rapid recurrence (4 months) and spread to the retroperitoneum, abdomen, pelvis, and abdominal wall. She was started on a regimen of gemcitabine and cisplatin and progressed on therapy. She was subsequently in our institution and on further review of personal and family history; it was revealed that both her two daughters had an early-onset hysterectomies (<age 30). The patient's two daughters had biopsy-confirmed cutaneous leiomyomas. Genetic counseling

and testing ensued, and she was found with a germline mutation in *fumarate hydratase* (*FH*). Her systemic therapy was switched to a combination of bevacizumab and erlotinib similar to a reported phase II trial. She had a partial response and remains on therapy for greater than 6 months. Lastly, her children have been referred for testing and screening and have been found to have the similar *FH* gene mutation.

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Part III

Surgical and Local Control Modalities

Surgical Approaches to Early-Stage Renal Cell Carcinoma

9

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Contents

This work was supported in part by Fox Chase Cancer Center via institutional support of the Kidney Cancer Keystone Program.

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Key Points

- The incidence of renal cell carcinoma (RCC) continues to grow, accounting for 5 and 3 % of all new cancer cases in 2014 in men and women, respectively.
- The gold standard treatment for localized RCC is surgical excision although ablative techniques and active surveillance (AS) have emerged as treatment alternatives in appropriately selected patients, even with larger tumors. Each treatment approach offers its own unique advantages and disadvantages.

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_9

- Partial nephrectomy provides equivalent oncologic outcomes to radical nephrectomy. Urologic oncologists must weigh the risks and benefits of improved renal functional outcomes with an increased risk of short-term morbidity associated with partial nephrectomy. In the appropriate setting, partial nephrectomy may be considered with both clinical T1 and T2 renal tumors.
- With the increasingly aging population, the importance of quantifying the risk of RCC-related death against the risks of patient's medical comorbidities has been recognized. Many nomograms now exist examining this risk/benefit equation.
- The RENAL nephrometry scoring system was the first standardized system introduced to objectify the salient features of a renal mass and can be used preoperatively to help predict tumor histology, grade, and perioperative outcomes.
- The importance of precisely measuring renal function by estimating a patient's glomerular filtration rate rather than relying on serum creatinine is paramount with expanding data showing the prevalence of chronic kidney disease as well as its negative impact on cardiovascular and overall health.
- This chapter outlines the objective tools available to arrive at an optimal treatment decision for each individual patient accounting for all the potential risks balanced against the benefits.

9.1 Preoperative Evaluation

The incidence of renal cell carcinoma (RCC) continues to rise due to the widespread use of cross-sectional imaging [1] with the greatest absolute increase noted in renal tumors sized 2–4 cm [2]. According to the National Cancer Institute, in 2014, there were an estimated 63,920 new cases of renal tumors, representing 5 and 3 % of all male and female cancers in 2014, respectively [3]. Additionally, in 2014, there were 13,860 estimated deaths from kidney cancer, accounting for 3 % of all male cancer-related deaths [3]. Survival for stage I and II RCC—T1 or T2 tumors without evidence of nodal or metastatic disease—has been reported at 96 and 82% [4]. These favorable survival rates are consistent with the AUA guidelines regarding the management and outcomes of the clinical T1 renal mass, which demonstrate that recurrence-free survival ranged from 87.0 % for ablative therapy to 99.2 % for surgical treatment of T1 renal masses [5]. Most new cases of localized RCC present incidentally as an enhancing renal mass [6]. Historical series demonstrate that 77–83.9 % of these lesions represent a malignant tumor of the kidney with clear cell carcinomas, the most common histologic subtype [7, 8].

According to the most recent National Cancer Comprehensive Network (NCCN) Guidelines, evaluation of a newly diagnosed renal mass consists of a complete history and physical examination, urinalysis, complete blood count, comprehensive metabolic panel including serum creatinine, contrast-based abdominal cross-sectional imaging if the mass was discovered on an ultrasound, chest x-ray, or CT scan of the chest. Abdominal MRI may be utilized when there is concern for renal vein and/or inferior vena caval involvement, or if an allergy or renal insufficiency prohibits the use of contrast dye [4]. Additionally, bone scan, brain MRI, and further metastatic workup are recommended if the patient has clinical signs or symptoms such as bone pain, an elevated alkaline phosphatase, or seizures [4]. Although not explicitly stated in the NCCN Guidelines, an estimation of the patient's glomerular filtration rate should be calculated because serum creatinine is a poor measure of renal function [9, 10]. Many patients who present with an enhancing renal mass have underlying chronic kidney disease (CKD) that is underrecognized using serum creatinine alone [11]. Furthermore, selective urinary cytology and endoscopic urinary tract evaluation should be performed in patients who have a history of urothelial cell carcinoma (UCC) of the bladder or upper

urinary tracts. Additionally, if the renal mass is central and UCC is suspected, cytology, endoscopic evaluation, and possibly biopsy should be employed to exclude a diagnosis of UCC of the renal pelvis, as this diagnosis would lead to a vastly distinct surgical treatment and follow-up.

Historically, the use of percutaneous renal mass biopsy (RMB) was limited to exclude the diagnosis of lymphoma, abscess, UCC, xanthogranulomatous disease, or metastatic cancer. Multiple studies have demonstrated that RMB is safe and potentially helpful in identifying benign lesions. A large study at the Mayo Clinic showed that approximately 30 % of lesions <4 cm removed by partial or radical nephrectomy were benign at final pathology [12]. These results are somewhat sobering when one considers that a recent meta-analysis has shown that the sensitivity and specificity of renal biopsy are 86–100 % and 100 %, respectively [13]. Thus, the pretreatment use of RMB may limit the incidence of surgery on benign renal masses. Nevertheless, the routine use of RMB may not be necessary; however, it can be most helpful in high-risk surgical patients or in patients in whom the radiologic characteristics of the renal mass are indeterminate or equivocal.

Once the evaluation of an enhancing renal mass has been completed, the urologic surgeon then needs to consider the risks of intervention against the biology of the disease and the patient's competing health risks. Although localized RCC is eminently curable by excision, surgery carries the risk of procedure-related complications as well as patient comorbidity-related complications. Since localized RCC has such excellent short and intermediate survival rates when treated and grows yearly at predictable rates when observed [14], one does not want to compromise the patient's quality/duration of life due to treatment-induced complications when treatment may not affect a patient's overall survival.

9.2 Competing Risks Analysis

All choices are made in the context of a risk-benefit balance, and healthcare decisions are no exception (Fig. 9.1). The decision to proceed to treatment in young, healthy patients with localized RCC is relatively straightforward, since even small oncologic risks are not acceptable in the face of a long life expectancy. Elderly and/or comorbid patients require a judicious clinical strategy, since in this population, medical comorbidities and non-renal malignancies that are yet to be diagnosed compete with kidney cancer as the primary cause of death. Furthermore, the potential negative impact on the patient's quality of life due to unintended medical/surgical complications must be accounted for in the treatment decision-making process.

The risk-benefit equation must be seriously considered when one realizes that the proportion of the US population who will be aged 65 years or older in 2030 is estimated to be 20 % [15]. Some authors have estimated that 60 % of cancers and 80 % of all cancer-related deaths in the United States occur in patients over the age of 65 [16]. Similarly, as patients age, they develop medical comorbidities that may be severe enough to impact their ability to receive or tolerate optimal cancer therapies [17]. Thus, the severity of a patient's comorbidity needs to be contextualized against the biologic behavior of the cancer.

Today, such decision making regarding risks and trade-offs in the management of localized RCC remain largely qualitative; however, clinically useful methods to quantitate risk are beginning to emerge. For instance, several comorbidity indices and scores have been proposed [18], and new approaches are steadily being introduced [19]. The Charlson Comorbidity Index (CCI) [20] is one of the best studied and most commonly employed methods for risk stratification [18]. The CCI incorporates 19 disease entities that include such ailments as cardiovascular, pulmonary, hepatic, and renal dysfunction. The degree to which each condition contributes to the index depends on that condition's calculated impact on mortality. Today, even in a busy clinical setting, the CCI can be rapidly calculated using web-based tools (e.g., http://www.medal.org/visitor/www/qhc/index.html).

Another potentially useful objective measure of a patient's risk with surgery is the preoperative measurement of a patient's "frailty." Originally

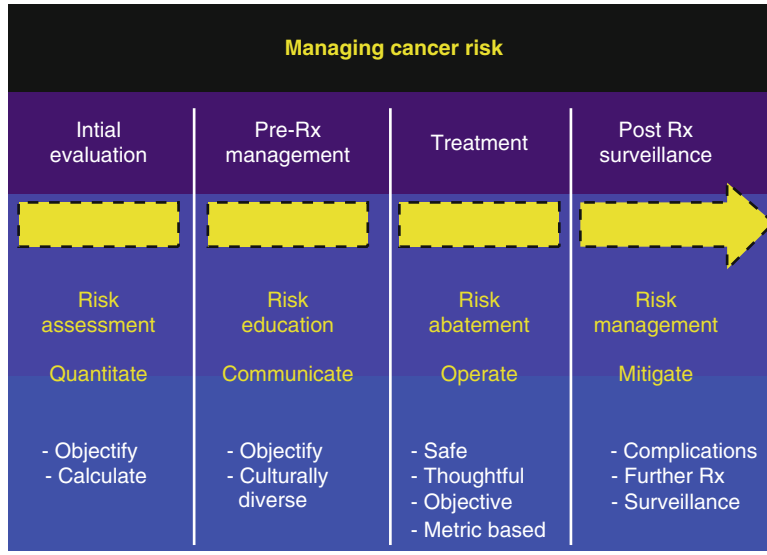

Fig. 9.1 Risk assessment algorithm for a patient with newly diagnosed localized renal cell carcinoma. Assessing risk occurs throughout the continuum of patient care. Risk assessment during initial evaluation requires quantitating treatment trade-offs in an objective manner. Pretreatment risk management requires education and communication about specific risks associated with a chosen therapy. Treatment risk management includes abatement of those risks during therapy using objective, metric-based data. Finally, posttreatment risk management involves mitigating future progression, complications, and anxiety using objective, data-driven strategies

developed by geriatricians, frailty is a relatively new concept that encompasses not only a patient's chronologic age but also a patient's ability to withstand physiologic stressors. Fried et al. initially introduced this concept and described frailty "as a biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes" [21]. In their initial study, Fried and colleagues operationalized the measurement of frailty focusing on the following domains: shrinking (weight loss or sarcopenia), weakness, slowness, poor endurance/exhaustion, and low activity. More recent studies have shown that the preoperative identification of intermediately frail or frail patients can predict for postoperative adverse events [22]. Ideally, in the future, an objective frailty score could be combined with other measures to develop a refined scale to precisely predict a patient's risk of adverse events with surgical intervention.

Also, in order to make informed and calculated decisions regarding the management of small localized renal masses, the physician must be able to estimate a patient's probability of dying from localized RCC and compare this to the patient's chances of dying from competing causes. Indeed, such predictive models have been developed for non-genitourinary solid malignancies [23, 24]. Similar tools are starting to emerge for localized RCC [25–27]. Our group recently developed a nomogram from a multivariable model based on over 30,000 patients from the Surveillance, Epidemiology, and End Results (SEER) program database who had resection of localized RCC [27]. The nomogram affords the clinician and the patient an opportunity to quantitate three competing 5-year mortality outcomes: (1) death from RCC, (2) death from other (non-RCC) cancers, and (3) non-cancer death. For instance, using the nomogram, a 75-year-old white male with a 4 cm tumor would have a 5-year mortality of 5 % from RCC versus 4.5 % from other cancers and 14 % from noncancerous causes. Even more recently, this nomogram has been adjusted to include a patient's comorbidities, using the CCI [28]. In light of these

	1 pt	2 pts	3 pts
(R)adius (maximal diameter in cm)		>4 but < 7	≥ 7
(E)xophytic/endophytic properties	≥ 50 %	<50 %	Entirely endophytic
(N)earness of the tumor to the collecting system or sinus (mm)	≥ 7	>4 but < 7	≤4
(A)nterior/Posterior	No points given. Mass assigned a descriptor of a, p, or x		
(L)ocation relative to the polar lines* * suffix "h" assigned if the tumor touches the main renal artery or vein	Entirely above the upper or below the lower polar line	Lesion crosses polar line	>50 % of mass is across polar line (a) or mass crosses the axial renal midline (b) or mass is entirely between the polar lines (c)

Fig. 9.2 RENAL nephrometry scoring system

competing risks and the known short-term indolent behavior of many localized RCC, active surveillance (AS) has emerged as a viable treatment strategy for patients with renal tumors. In this situation, RMB may prove beneficial in objectively evaluating the histology and Fuhrman grade in an attempt to more accurately predict the behavior of the mass.

When considering AS as a management strategy for a newly diagnosed renal mass, it is helpful to consider absolute, relative, and elective indications. Absolute indications include patients in whom surgery poses an immediate and unacceptable risk of mortality. Relative indications for observation include concomitant diseases, such as a second malignancy and/or significant but not overriding medical comorbidities. Lastly, some patients may simply wish to undergo a period of AS despite being low-risk surgical candidates. This constitutes an elective indication for AS and requires the treating physician to inform the patient of the available data on renal tumor growth kinetics, with limitations and the uncertain long-term risk of progression. No matter what the indication for AS of a renal mass, it must be understood that the patient and physician are both taking a calculated risk due to the heterogeneous and occasional unpredictable behavior of RCC.

In summary, quantification of a patient's perioperative risk as well as competing risks of death must be thoughtfully integrated into clinical decision making. Current ubiquitous qualitative approaches must be replaced by quantitative strategies. Given the known yearly growth rates of SRMs [14] and the low likelihood of developing metastatic RCC in masses less than 4 cm when followed for 24–30 months [14, 29], AS is a reasonable treatment strategy in the elderly or patients with severe medical comorbidities.

9.3 Objectification of Renal Tumor Anatomy

Despite, or because of, the myriad treatment options available to the patient and treating urologist, clinical decision making for localized RCC is overly subjective. It is based on numerous often qualitative factors, including competing health risks (real or perceived), the interpreted tumor anatomy, physician experience and comfort, and patient preference/perceptions of the ease/efficacy of various treatment modalities.

We introduced the RENAL nephrometry scoring system as a means to objectify the salient anatomic features of a renal mass on cross-sectional imaging. This system may be utilized to compare outcomes and develop metrics for treatment decision making [30] (Fig. 9.2). In the absence of a common nomenclature to describe the anatomical attributes of a renal tumor, treatment decision making is subject to an unmeasured physician's biases and individual experience. A tumor's nephrometry score is a structured and quantifiable method to describe the tumor's relevant anatomical features as they relate to the complexity of a tumor, its difficulty of resection, and potential treatment risks.

The scoring system is based on the five most reproducible features that characterize the

anatomy of a solid renal mass: (R)adius (scores tumor size as maximal diameter), (E)xophytic/endophytic properties of the tumor, (N)earness of the deepest portion of the tumor to the collecting system or renal sinus, (A)nterior (a)/posterior (p) descriptor, and (L)ocation relative to the polar line. All components except for the (A) descriptor are scored on a 1-, 2-, or 3-point scale. The (A) describes the principal mass location to the coronal plane of the kidney. The suffix "x" is assigned to the tumor if an anterior or posterior designation is not possible. An additional suffix "h" is used to designate a hilar location of the tumor (abutting the main renal artery or vein).

The RENAL nephrometry scoring system represents the first-introduced method to attempt to standardize the reporting of the salient anatomy of an enhancing renal mass. Subsequently, the PADUA score was introduced as another objective method to describe the anatomical features of a renal mass [31]. The PADUA score is remarkably similar to nephrometry with the exception of "the definition of the sinus lines and the evaluation of the anatomical relationship between the tumor and urinary collecting system or renal sinus" [31]. Lastly, the C-Index Method was introduced to characterize a tumor's centrality. This method requires a complex geometric calculation using cross-sectional imaging to determine the distance from the tumor center to the center of the kidney [32]. We believe that the RENAL nephrometry scoring system is unique in that it is an accessible system that can be learned rapidly and applied that reliably describes the most salient renal mass features.

By creating a reproducible system based on the renal mass anatomy, we have objectified the descriptions of renal masses that previously were simply referred to in terms such as "simple" or "difficult," thereby creating a platform to ascertain the optimal surgical approach. For example, in a recent evaluation of our institutional database, 94 % of low-complexity (nephrometry score=4–6) masses were treated with a PN, most using an MIS technique. Nephrometry has several additional uses beyond aiding in surgical treatment decision making. Recent investigators have adopted nephrometry to examine its ability to predict for functional, perioperative, and pathologic outcomes. Cha et al. showed that patients with higher "nephrometric variables," (R) and (E), were more likely to experience postoperative renal impairment after a MIS-PN [33]. Two other groups have shown that higher nephrometry scores predict for increased blood loss and longer ischemia time when undergoing either MIS-PN or open PN [34, 35]. Additionally, a prospective study at our institution validated that higher nephrometry scores can be used to predict prolonged warm ischemia time [36]. Finally, despite prior work reporting no significant biological differences between centrally and peripherally located tumors [37], nephrometry was recently evaluated to determine its ability to preoperatively predict the histology and grade of enhancing renal masses. In this work, the authors found a high correlation between nephrometry score and tumor grade ($p<0.0001$) and histology ($p<0.0001$) [38]. Specifically, papillary RCCs had the lowest total nephrometry score, while clear cell RCCs had higher nephrometry scores. Furthermore, benign lesions tended to be smaller, more endophytic, and non-hilar [38].

Nephrometry creates a platform to standardize salient renal mass anatomy. In doing so, objective treatment decision making can be performed when the urologist considers the functional, perioperative, and preoperative pathologic information that one can derive from the RENAL nephrometry scoring system.

9.4 Assessment and Implications of Chronic Kidney Disease (CKD)

The systematic review by the RCC guidelines committee of the AUA highlights the priority of goals when managing localized RCC: (1) optimize cancer treatment, (2) preserve renal function, and if the first two goals are met, (3) utilize a minimally invasive technique while minimizing the risk of adverse postoperative events [5]. Published series have established the oncologic efficacy of nephron-sparing surgery (NSS) for pT1a and pT1b renal tumors [5, 39–42]. Despite these findings and other data indicating that PN confers a non-oncological survival advantage,

nationally the use of PN for tumors less than 4 cm continues to be less than 30 % [43]. As more incidental renal masses continue to be detected and the adverse relationship between long-term CKD and morbidity/mortality is uncovered, the importance of renal functional preservation continues to be paramount.

Traditionally, serum creatinine (sCr) has been used to measure the presence or absence of renal dysfunction; however, this can be a misleading value, since sCr can be affected by age, gender, muscle mass, and diet. Furthermore, since creatinine is both secreted and reabsorbed by renal tubules, certain medications, such as cimetidine and sulfonamides, can alter sCr by inhibiting its tubular secretion. Recent data suggest that serum creatinine measurements are a poor tool to estimate the degree of renal impairment [9, 10]. In fact, in a recent cross-sectional analysis comparing the National Health and Nutrition Examination Surveys (NHANES) between 1988–1994 and 1999–2004 consisting of approximately 29,000 patients, 25 % of patients with a "normal" sCr had chronic kidney disease (CKD) stage 3 or greater, as defined by the National Kidney Foundation [44]. With the recent data underscoring the prevalence of CKD in the general population, attention has focused on estimating the glomerular filtration rate (GFR) as a measure of a patient's renal function. More precise measures of GFR have recently been adopted including the MDRD and CKD-EPI. Therefore, the socioeconomic and health implications of significant national underutilization of NSS are likely clinically underestimated.

The risk of postoperative chronic kidney disease after RN when compared to PN has been well studied. McKiernan et al. showed that the risk of having a postoperative baseline sCr greater than 2.0 mg/dL was significantly greater following RN when compared to a PN [45]. A more precise quantification of CKD after nephrectomy was undertaken by Huang et al. Using the MDRD equation to estimate GFR, the authors found in a multivariable analysis that RN was an independent risk factor for patients developing an eGFR of less than 60 ml/min and less than 45 ml/min [46]. The incidence of baseline renal dysfunction (eGFR <60) in their study was 26 %.

The relationship between CKD and the risks of death, cardiovascular events, and hospitalization rates is clinically relevant but has previously not received much attention because it is often an event that occurs well past the initial surgical loss of nephrons. With each 15 ml/min diminution of eGFR below 60 ml/min, the risk of death, cardiovascular events, and hospitalization increases [47]. For example, the adjusted hazard ratio for death in a patient with an eGFR of 45–59 ml/min is 1.2 while it is 5.9 for an eGFR less than 15 ml/min [47]. Furthermore, the interaction between age and CKD and their effects on survival requires the urologist to diligently assess an elderly patient's renal function preoperatively. In one study, more than 50 % of patients older than 75 years died within 2 years after starting dialysis [48]. The median survival time for this aged population on dialysis was 22 months.

The prevalence of CKD stage III or higher based on NHANES 1999–2004 data has increased to over 8 % [44]. It is unclear if a population enriched for patients with radiographically concerning RCC reflects this trend or has a potentially higher risk of CKD. In a recent review of our institutional kidney cancer database, we showed that although 88 % of all patients presenting for surgery with a solid renal mass at our institution had a "normal" sCr ($\leq$1.4 mg/dl), 12.5 % of these patients had CKD stage III when estimating GFR [11]. Moreover, 23 % of patients 70 years old or greater with a seemingly normal sCr had CKD stage III. These findings support reports by other authors who have argued for more precise measurement of a patient's renal function, either by the MDRD equation or the newly developed Chronic Kidney Disease-Epidemiology Study equation, to better assess a patient's renal function [9, 10]. Finally, the national average of NSS, ranging from 27 % [49] to 40 % [43] for pT1a tumors, is concerning in light of our findings showing an underestimation of chronic kidney disease by routine serum creatinine monitoring. This study highlights the fact that both eGFR and CKD stage must be routinely calculated and clinical decisions based on these variables not sCr, especially in the elderly.

9.5 Treatment of Early-Stage RCC: Excise

9.5.1 Comparison of Oncologic Outcomes Between Radical Nephrectomy and Partial Nephrectomy

The mainstay of treatment for RCC is surgical therapy due to its resistance to chemotherapy and radiation therapy. Recent advances in the development of targeted therapies for advanced RCC have resulted in longer survival for patients with metastatic RCC; however, treatment for localized RCC remains surgical extirpation. The management of RCC has been governed by Robson's initial description in 1963 of a radical nephrectomy (RN) for the treatment of all renal tumors [50]. Utilizing a flank, subcostal, or midline incision, Robson's description of an RN included the removal of the entire kidney, perirenal fat, surrounding Gerota's fascia, overlying peritoneum, and adrenal gland [50]. This approach resulted in excellent oncologic outcomes [51]. In cases where surgical extirpation of the kidney would render a patient functionally or anatomically anaphoric, an "essential" partial nephrectomy (PN) was performed in these select patients to avoid the need for renal replacement therapy. As data on oncologic outcomes of patients who underwent an "essential" PN emerged, the use of PN for elective indications was gaining acceptance. During the past decade, the paradigm has shifted toward treating localized RCC with nephron-sparing surgery (NSS) as oncologic outcomes have proven to be equivalent to traditional RN (Table 9.1). In fact, in the recent AUA guidelines, which reviewed all the existing literature for oncologic outcomes for RN and PN, recurrence-free survival rates were equal at 98.0–99.2 %, respectively [5].

In addition to oncologic equivalency, nephron preservation also results in improved renal functional outcomes after surgery [45, 46]. Furthermore, several recent studies have shown a defined benefit with PN compared to RN in terms of overall survival and reduced rates of cardiovascular events and non-cancer-related deaths [52–54]. Weight et al. published the Cleveland Clinic's follow-up data comparing survival outcomes in patients undergoing RN or a PN for a cT1b renal mass. In this cohort of 1,004 patients, postoperative eGFR was an independent predictor of overall survival and cardiac-specific survival on multivariate analysis. Patients treated with PN had a statistically significant improved 5-year OS compared to patients treated with RN (85 % vs. 78 % ($p=0.01$)) [52]. Interestingly, of the 175 deaths in this cohort, 48 were due to cardiovascular events and 19 were related to renal failure. Similar conclusions were reached by Thompson et al. and Huang et al. when examining the Mayo Clinic nephrectomy registry as well as the SEER cancer database. Their data demonstrated that in patients younger than 65 years old treated for a pT1a renal mass, RN was significantly associated with death from any cause (RR 2.16, $p=0.02$) [53]. Also, a query of the SEER cancer registry showed a statistically significant increase in the risk of cardiovascular events ($p<0.05$) and all-cause mortality (HR 1.46, $p<0.001$) for patients treated with RN for a pT1a renal mass [54]. Furthermore, in a graded fashion, renal dysfunction has been shown to be associated with significantly increased cardiovascular risks, hospitalizations, and mortality [47]. Finally, when employed in elective situations, health-related quality of life scores were higher in the PN compared to RN group [55] with equivalent lengths of stay and direct hospital costs [56].

Despite oncologic equivalency and improved renal functional outcomes, NSS carries a higher risk of a major urologic complication that must be considered in the risk/benefit equation. In the recent AUA guidelines concerning the management of the clinical T1 renal mass, the complication rate for open PN ranges from 4.5 to 8.7 % based on the results of 15 published studies [5]. Also, the recent EORTC trial comparing PN to RN in tumors less than 5 cm highlights this risk/benefit balance. In this prospective randomized study of 541 patients, PN was associated with a statisti-

Table 9.1 Oncologic outcomes in patients treated with radical nephrectomy compared to nephron-sparing surgery

Series	Year	Tumor stage	No. patients/average tumor size (cm)		% 5-year CSS		Controlled for
			RN	NSS	RN	NSS	
Robson	1969	Stage 1 (confined to the kidney)	33	n/a	66	n/a	
Butler	1995	T1a	42 (2.7 ± 0.8)	46 (2.5 ± 0.8)	97	100	Age, gender, tumor size, location and stage, renal function, comorbidities
D'Armiento	1997	T1a	21 (3.21)	19 (3.34)	96	96	Age, gender, date of surgery, tumor size, grade
Indudhara	1997	T1a, T1b	71	35	94	91	Age, gender, date of surgery, tumor size, stage
Belldegrun	1999	T1a, T1b	125 (6.2)	108 (3.6)	91.2	98	Age, gender, date of surgery, tumor stage, follow-up
Barbalias	1999	T1a, T1b	48 (3.8)	41 (3.5)	98.4	97.5	Age, tumor size, stage, location
Lee	2000	T1a	183 (3.0)	79 (2.5)	95	95	Gender, tumor histology, stage, age, gender, date of surgery, tumor stage, grade, location
Thompson	2009	T1b	704 (5.5)	239 (5.0)	91	96	Age, gender, local or systemic symptoms at presentation, Charlson comorbidity index, diabetes, solitary kidney, preoperative serum Cr, eGFR, CKD, 2002 primary tumor classification
Crepel	2010	T1a	5,658 (2.8)	1,622 (2.5)	97.5	98.2	OCM (other cause mortality), age, year of surgery, tumor size, and Fuhrman grade

cally significant increased risk of severe hemorrhage, defined as >1 L, and urinary fistulas ($p < 0.001$) [57]. Conversely, patients who underwent a PN had a statistically significant lower sCr at follow-up ($p < 0.0001$). Similarly, other studies have shown that as tumor size or tumor complexity increases, the incidence of technical adverse events increases too. Patard et al. compared morbidity in patients undergoing PN for tumors <4 cm and >4 cm. In this study, there was a statistically significant increase in the rates of blood transfusions ($p = 0.001$) and urinary fistula ($p = 0.01$) in patients undergoing PN for tumors >4 cm [58]. Clearly, the risks of chronic kidney disease and their attendant detrimental health effects need to be quantified and weighed against the more immediate and short-term surgical risks.

Table 9.2 Oncologic comparison between open and laparoscopic partial nephrectomy

		5-year disease-free survival (%)			
Series	No. patients	T1a	T1b	T2	Positive margins (%)
Fergany et al. (2000)	107	97.6	95	100	–
Gill et al. (2002)	200	91 vs. 73	9 vs. 27	N/A	3.0 vs. 1.0
Permpongkosol et al. (2005)	143	91.4 vs. 97.2	75 vs. 75	N/A	2.4 vs. 1.7
Gill et al. (2007)	1,800	99.3 vs. 99.2		N/A	2.9 vs. 1.3

9.5.2 Partial Nephrectomy for pT2 Renal Masses

Extending the partial nephrectomy experience for pT1b tumors, newer series have demonstrated that PN for T2 renal masses has equivalent oncologic outcomes compared to RN with acceptable perioperative complication rates. Hansen et al. published a study examining the SEER database which compared 245 patients who underwent a PN and 8,602 patients who underwent an RN for a renal mass >7 cm. In their analysis, the cancer-specific mortality (CSM) for an RN was 5.7 % and 17.7 % at 2 and 5 years, respectively [59]. For PN, the CSM was 3.4 and 11.9 % at the same time points which was not statistically significant different [59]. Breau et al. found that RCC-specific survival and OS were similar when their institution matched PN to RN for stage T2 tumors or greater. Local recurrence occurred in 4 (6 %) patients, while distant spread occurred in 15 (22 %) individuals [60].

Similarly, our institution performed an analysis of 49 renal tumors >7 cm that were treated by PN. The 5- and 10-year RCC-specific survival was 94.5 and 70.9 % [61]. In our series, 8.2 % of patients required blood transfusions, and 12.2 % developed urinary fistulas [61]. As previously mentioned, in a recent EORTC trial, 541 patients with cT1 renal masses ≤5 cm were randomized to PN or RN. PN was associated with a statistically significant increased risk of severe hemorrhage (defined as >1 L) and urinary fistula ($p < 0.001$) [57]. Conversely, patients who underwent a PN had a statistically significant lower serum creatinine at follow-up ($p < 0.0001$). Our study, among others, has shown that in the appropriate setting, PN for pT2 masses is oncologically sound with acceptable complications rates. Furthermore, as expected, PN is associated with better renal functional preservation. Urologists must weigh the risks and benefits of PN, including improved renal functional outcomes and improved cardiovascular status, with an increased risk of short-term morbidity in the setting of oncologic equivalence.

9.5.3 Comparison of Open and Minimally Invasive Techniques in the Treatment of Localized RCC

With the advent of minimally invasive surgery, laparoscopic techniques have been applied to the kidney. There was an initial reluctance to adopt laparoscopic renal surgery widely because of concerns for tumor seeding of the peritoneum. Also, maculation of specimens raised concerns for inadequate staging. Today, nephrectomy specimens are removed intact, and concerns over tumor seeding have not been substantiated. Indeed, although prospective randomized trials of open versus laparoscopic radical nephrectomy were never completed, long-term retrospective data suggest oncological equivalence between the two approaches [62–66] (Table 9.2). Today, given significantly lower intraoperative blood loss and shorter convalescence, laparoscopic RN is the standard of care for renal surgery that requires total removal of the kidney [62].

In 1990, the first laparoscopic radical nephrectomy (LRN) was performed by Clayman et al. for a 3 cm oncocytoma [67]. In that case report, each segmental artery was dissected and individually ligated, because the clips available at that time

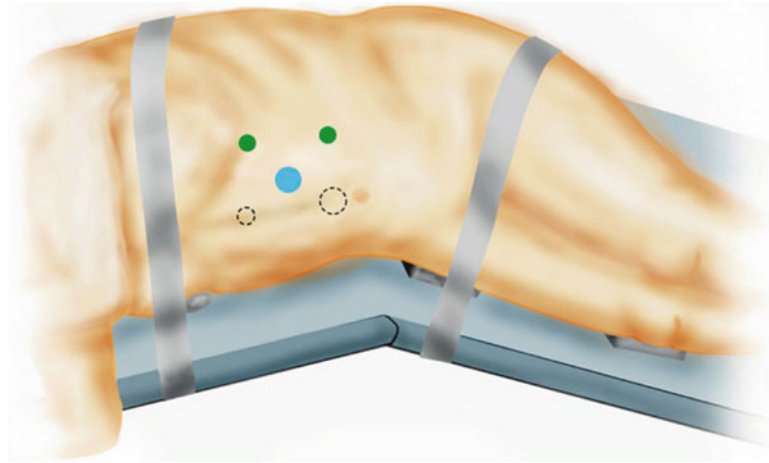

Fig. 9.3 Usual port site arrangement for left transperitoneal robot-assisted partial nephrectomy. Two common arrangements are depicted. *Blue circles* indicate camera ports. *Dashed circles* indicate assistant ports. *Larger circles* represent 12 mm ports, while the *smaller circles* represent 5 mm ports. *Green circles* represent 8 mm ports that accommodate the robotic arms

were not large enough to secure the main renal artery. Furthermore, a preoperative angioinfarction of the kidney was performed, and intraoperatively, a ureteral catheter was placed. Since that initial report, the laparoscopic renal surgery rapidly gained traction. Presently, at centers of excellence, the vast majority of nephrectomies are performed via a laparoscopic approach. Furthermore, surgery for large renal tumors and tumors with thrombi extending into the renal vein and even the vena cava is now being performed laparoscopically [68–70].

Coincident with the growth of laparoscopy has been the increased detection of incidental SRMs during the last two decades, as cross-sectional imaging has become a routine diagnostic tool [1]. Thanks to the widespread acceptance of NSS and refinement of laparoscopic instrumentation, a patient can be offered a PN via laparoscopic approaches (with and without robotic assistance) utilizing only three or four small incisions, none measuring greater than 1.2 cm. A large multi-institutional retrospective study comparing laparoscopic partial nephrectomy (LPN) with OPN provided evidence on multivariate analyses that LPN was associated with decreased blood loss and shorter operative times and hospital stays [71]. However, perioperative/postoperative complications, such as prolonged warm ischemia and renal hemorrhage, and re-exploration rates were notably higher in the LPN group, while oncologic control appeared to be equivalent in the two groups. The AUA systematic review published its guidelines on the treatment for stage I renal tumors identifying a nearly 50 % increase in "major complications" in LPN compared to OPN [5]. Despite the increase in major urologic complications, cancer control for appropriately selected patients appears to be preserved [72].

More recently, robotic-assisted laparoscopy has emerged as another tool in the armamentarium for treatment of localized kidney cancer (Fig. 9.3). As urologists have become more familiar with robotic techniques, the usage of robotics has broadened to include NSS. Robotic assistance enables the surgeon to perform more efficient intracorporeal suturing and thus safely resect larger, more anatomically complex lesions. Furthermore, the learning curve for robotically assisted laparoscopic partial nephrectomy (RALPN) may be less steep than LPN, based on equivalent same surgeon results when comparing initial RALPN versus vast LPN experience [73]. Sitting at the console, the robotic user can rotate one's wrists 180° and pass suture from virtually any angle. Renal reconstruction can be performed in 3-D, and the passing of suture through the kidney is easier than with pure laparoscopic technique due to the wrist motions of the robot.

Many small series have been published showing that a RALPN is technically feasible without increasing patient morbidity [74–77] (Table 9.3). These series do not have long enough follow-up to show equivalent oncologic control as the open

Table 9.3 Short-term outcomes of published robotic-assisted partial nephrectomy series

Series and institution	RAPN (n)	Tumor size (cm)	Complications by Clavien Grade (II–V)	Positive margins (n)	Urine leaks (n)
Gettman et al. Mayo Clinic	13	3.5	None	1	NR
Kaul et al. Henry Ford	10	2	II: 1 III: 1	1	1
Caruso et al. New York University	10	1.95	III: 1	0	NR
Rogers et al. National Institutes of Health	8	3.6	None	0	NR
Aron et al. Cleveland Clinic	12	2.4	II: 2 III: 1	0	0
Deane et al. UC Irvine	11	2.3	III: 1	0	NR
Ho et al. Medical University of Innsbruck, Austria	20	3.5	None	0	0
Wang et al. Washington University	40	2.5	II: 2 III: 1 Undefined: 4	1	1
Michli et al. Cooper University Hospital	20	2.7	II: 1 III: 1	0	NR
Gong et al. City of Hope	29	3.0	NR	0	NR
Benway et al. Multiple institutions	129[a]	2.9	II: 1 III: 4 Undefined: 6	5	3
Scoll et al. Fox Chase Cancer Center	100	2.8	II: 5 III: 5 V: 1	5	2
Total	**402**	**1.95–3.6**	**II: 12** **III: 15** **V: 1** **Undefined: 10**	**13 (3.2 %)**	**7**

NR denotes data not reported
Undefined indicated cardiopulmonary, thromboembolic, and bleeding complications that cannot be graded from the reported descriptions
[a]Multi-institutional cohort includes updated data from previously published single-institution series

or laparoscopic approaches; however, currently, there is no suspicion that the technique is inferior [74–76]. The largest recent series concluded that RALPN is an oncologically sound approach with acceptable immediate nephron-sparing outcomes [77].

Finally, due to its location, the kidney can be accessed via a pure retroperitoneal approach (Fig. 9.4), and retroperitoneoscopic renal surgery was first described in the early 1990s. This approach offers rapid and direct access to the hilum [78]. However, the retroperitoneoscopic approach is unfamiliar to some urologists, and the small working space can make the operation difficult and tedious, especially in patient with copious retroperitoneal fat, which can impede visualization. Finally, the retroperitoneum, especially in the presence of copious fat, lacks reliable landmarks that a transperitoneal approach offers. This absence of predictable anatomical cues contributes to a steep learning curve and may lead to catastrophic complications in inexperienced hands. In one multi-institutional

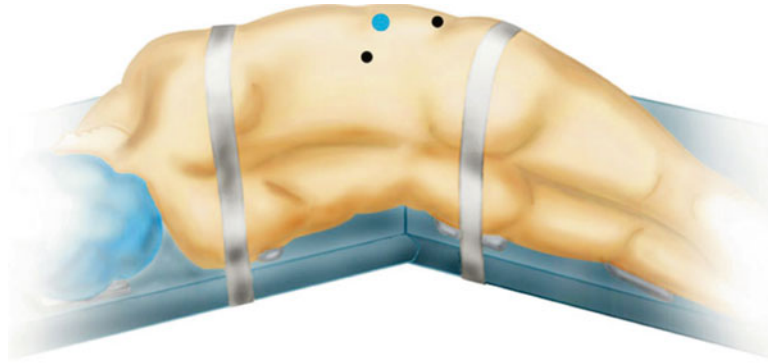

Fig. 9.4 Usual port site arrangement for right retroperitoneal laparoscopic radical nephrectomy. *Blue circle* indicates camera port. *Larger circle* represents 12 mm port, while the *smaller circles* represent 5 mm ports

report, the IVC was transected in two patients with a stapling device, because it was mistaken for the main right renal vein [79]. Nonetheless, there are clinical scenarios where this approach may be more advantageous. In morbidly obese patients and those with prior extensive abdominal surgery or radiation, a retroperitoneal approach can be safely performed without significant increases in morbidity, blood loss, or operative time [80]. In prior series looking at head-to-head comparisons between transperitoneoscopic and retroperitoneoscopic LPN, reported clinical outcomes were comparable in terms of blood loss, operative times, and convalescence [81]. Despite these favorable results, this technique does require an additional level of expertise.

As indications expand and surgical skills become more refined, the pendulum has gradually swung away from open and toward MIS for kidney cancer, especially at centers of excellence. Due to the stage migration associated with RCC in recent years, the historic standard of open radical nephrectomy is unwarranted, and the associated CKD is preventable and potentially harmful [82]. Assuming equivalent oncologic outcomes and renal preservation, minimally invasive techniques should be employed to minimize patient morbidity [5].

9.5.4 Role of Lymph Node Dissection in Low-Risk RCC

The role of a lymph node dissection (LND) in RCC, especially low-risk RCC, is unproven. EORTC 30881 was a large prospective phase 3 trial that randomized 772 patients with cTanyN0M0 to RN +/− LND. Seventy percent of these patients had pT1-2 disease [83]. There were no statistical differences between RN with LND versus RN alone with respect to morbidity, progression-free survival, time to progression, and overall survival [83]. Importantly, only 1 % of these patients had pathologic node positive disease which was non-palpable at surgery and not evident on preoperative imaging. Conclusions of this study show that patients with low-risk RCC (cT1-2N0M0) most likely do not benefit from LND [83]. However, this study may have been underpowered to detect a significant difference in oncologic outcomes in patients with low-risk RCC.

9.6 Treatment of Early-Stage RCC: Ablation

9.6.1 Cryoablation Versus Radiofrequency Ablation (RFA)

The diagnosis of localized RCC continues to increase with the widespread use of cross-sectional imaging for unrelated reasons [1], and localized RCC or small renal masses (SRMs) may account for as much as two-thirds of newly diagnosed RCC [84]. Ablative techniques in the form of cryoablation or radiofrequency ablation (RFA) are attractive treatment modalities for elderly patients or patients with significant medical comorbidities because they are either percutaneous or minimally invasive, thus poten-

tially avoiding the risks of both general anesthesia and major surgery. The recent AUA guidelines for the clinical T1 renal mass included the results of ablative techniques, which encompassed 34 studies with 1,389 patients undergoing either cryoablation or RFA. Recurrence-free survival rates for cryoablation and RFA were 90.6 and 87.0 %, respectively [5]. Major urological complications occurred in 4.9 and 6.0 % of cryoablation and RFA cases [5].

Cryoablation results in tumor destruction by inducing rapid freeze-and-thaw cycles [85]. Initial ice formation results in a number of physiological and mechanical cellular disruptions, including protein denaturation and cellular membrane disruption, ultimately leading to tumor kill [85]. RFA relies on the conversion of radiofrequency waves to heat, resulting in thermal tissue damage [86]. Similar to cryoablation, RFA results in tumor destruction by protein denaturation and cellular membrane disruption.

A recent meta-analysis comparing cryoablation to RFA of 47 series totaling 1,375 renal tumors found that intermediate oncologic efficacy may favor cryoablation [87]. In this study, the authors found that patients undergoing RFA more often required a repeat ablative session ($p<0.0001$) as well as having a higher rate of local tumor progression ($p<0.0001$) [87]. The higher incidence of local tumor progression occurring with RFA was confirmed on univariate ($p=0.001$) and multivariate ($p=0.003$) analysis [87]. Finally, there was a higher incidence of progression to metastatic disease with RFA (2.5 % vs. 1 %); however, this did not achieve statistical significance ($p=0.06$) [87].

These findings were consistent with another meta-analysis comparing excision, ablation, and observation of the small renal mass. In this study of 99 series including 6,471 renal tumors, the authors found a local recurrence rate of 4.6 % after cryoablation and 11.7 % after RFA [88]. When compared to surgical excision, multivariate analysis revealed a significantly higher incidence of recurrence with cryoablation (RR = 7.45) and RFA (RR = 18.23) [88]. No significant difference was seen between cryoablation and RFA for the development of metastatic disease. Finally, a retrospective study from the Mayo Clinic compared PN, percutaneous RFA, and percutaneous cryoablation for cT1 renal masses. For patients with cT1a tumors, recurrence-free survival was similar among all three treatments [89]. However, metastasis-free survival was significantly improved with PN ($p=0.005$) and cryoablation compared to RFA ($p=0.021$) [89]. For patients with cT1b tumors, local recurrence-free survival ($p=0.81$) and metastasis-free survival ($p=0.45$) were similar between PN and cryoablation [89]. Patients with cT1a and cT1b tumors who underwent PN had a significantly improved overall survival ($p<0.001$) compared to patients who underwent ablation at 5 years of follow-up, potentially reflecting a patient selection bias due to medical comorbidities [89].

9.6.2 Percutaneous Versus Laparoscopic Approach to Ablation

Recently, ablative techniques for renal tumors have moved toward the use of cryoablation rather than RFA. Cryoablation can be performed both surgically—open or laparoscopically—and percutaneously. Theoretically, surgical cryoablation offers direct placement of cryotherapy probes and allows for real-time visual and continuous monitoring of ice ball formation and extension; however, surgical treatment subjects the patient to the risks of general anesthesia as well as the inherent risks of surgery. Percutaneous cryoablation has the potential advantages of improved patient tolerance, faster recovery, avoidance of general anesthesia, and lower periprocedural risks. Prior comparisons between the two approaches have focused on pain requirements and length of stay [90].

A recent meta-analysis of the literature was performed comparing the oncologic outcomes of surgical and percutaneous cryoablation of localized RCC. In this review, 42 studies including 1,447 renal lesions were pooled and analyzed. There was no significant difference in patient age, tumor size, or duration of follow-up between surgical and percutaneous cryoablation [91]. The

rates of residual tumor ($p=0.24$) and recurrent tumor ($p=0.44$) were not statistically significant between surgical and percutaneous cryoablation [91]. In the reported literature, there were only two reports of the development of metastatic disease in the surgical group and one report in the percutaneous group [91]. Based on these findings, the authors concluded that neither approach was superior.

9.7 Treatment of Early-Stage RCC: Observation

9.7.1 Growth Rates

Overdiagnosis of malignancy, along with receipt of unneeded treatment as well as its attendant risks, is arguably the most important harm associated with early cancer detection. Recent attention has been directed toward describing the natural history, or growth kinetics, of localized RCC under observation in an effort to identify which lesions are safe to observe and which require early definitive intervention. In an attempt to consolidate these individual small experiences and identify growth trends in SRMs, Chawla et al. performed a meta-analysis of nine single-institution retrospective series including 234 masses followed for a mean duration of 34 months. Initial tumor diameter was 2.6 cm, mean growth rate was 0.28 cm/year, and pathologic confirmation was available in 46 % (92 % RCC or RCC variant) [14]. We have recently updated these findings in a pooled analysis of 259 patients (284 masses) with available individual level data [29]. This analysis revealed a mean age of 66.9 years, a mean initial tumor size of 2.4 cm, and mean final tumor size of 3.2 cm. With a mean duration of observation of 33.6 months, the calculated mean change in maximal diameter per year (linear growth rate) was 0.33 cm/year. These data confirm initial observations that a majority of localized renal tumors exhibit slow radiographic growth with low metastatic potential while under an initial period of observation.

Although growth kinetics of small renal masses were initially studied in cT1a masses, there now exists an emerging literature examining growth rates of cT1b and cT2 tumors that have been followed with active surveillance. In this recently published study with 39 months of follow-up, the mean linear growth rate was 0.44 cm/year, and 66 % of these patients continued with AS while 34 % progressed to definitive intervention [92]. Specifically, tumors that were continued on AS had a growth rate of 0.37 cm/year, while masses that underwent definitive surgical management grew at 0.73 cm/year [92]. Importantly, no patients in this study developed metastatic disease after AS. This study appears to suggest that the growth kinetics of even larger renal masses are predictable and may safely be monitored using an active surveillance protocol [92].

9.7.2 Progression Rates

Progression to metastatic disease in patients with localized RCC or SRMs under AS is uncommon and poorly documented in the literature. Our recent systematic review identified 18 patients progressing to metastatic disease from a cohort of 880 patients with SRMs under AS (a total of 2.1 %) [29]. Comparing patients that progressed to metastatic disease in our systematic review ($n=18$) with those that did not in our pooled cohort of patients with individual level data ($n=281$), the duration of observation was similar between groups (40.2 vs. 33.3 months; $p=0.47$), but there were significant differences in mean patient age (75.1 vs. 66.6 years; $p=0.03$). Trends in patients progressing to metastases included larger tumor size (4.1 vs. 2.3 cm; $p<0.0001$) and estimated tumor volume (66.4 vs. 15.1 cm^3; $p<0.0001$) at diagnosis as well as mean linear (0.80 vs. 0.30 cm/year; $p=0.0001$) and volumetric growth rates (27.1 vs. 6.2 cm^3/year; $p<0.0001$). Important observations to consider are that metastasis was a late event (>3 years following diagnosis), all lesions that progressed were >3 cm at the time of metastasis, all demonstrated positive growth rates, and no lesion exhibiting zero net growth while under surveillance has developed metastases while under observation.

9.8 Follow-Up for Clinically Localized RCC

In 2013, the American Urological Association released guidelines for the follow-up and surveillance of clinically localized RCC managed by AS, partial nephrectomy, radical nephrectomy, or ablative techniques. Abdominal CT, US, or MRI should be preformed within 6 months of active surveillance initiation to establish growth rate and continued imaging at least annually thereafter [93]. Other imaging studies should be ordered as clinically indicated.

For renal masses treated with an ablative technique, abdominal cross-sectional imaging, with and without IV contrast, unless contraindicated, should be performed at 3 and 6 months postoperatively with continued imaging annually for 5 years. Imaging beyond 5 years is optional based upon individual patient risk factors. Biopsy should be performed pretreatment and again for the following indications: if there is new enhancement, an interval progression in size of an ablated tumor or tumor bed with or without enhancement, new nodularity in or around treatment zone, failure of treated lesion to regress over time, or satellite or port side lesions [93].

After partial nephrectomy for low-risk disease (pT1, N0, Nx), a baseline abdominal CT or MRI should be performed 3–12 months following surgery. If the initial postoperative scan is negative, abdominal imaging may be performed annually for 3 years based upon patient risk factors. After radical nephrectomy for low-risk disease, again baseline imaging should be preformed 3–12 months following surgery. If the initial postoperative imaging is negative, imaging after 12 months is at the physician's discretion. After both radical and partial nephrectomy for low-risk disease, the patient should undergo an annual chest x-ray for 3 years and then again as clinically indicated [93].

For moderate- to high-risk disease (pT2-4N0 Nx or any stage N+), baseline abdominal imaging should be performed 3–6 months following surgery with continued imaging every 6 months for at least 3 years and annually through year 5. Beyond 5 years, imaging is at physician's discretion. Baseline CT chest is within 3–6 months following surgery with continued CXR or CT every 6 months for 3 years then annually through year 5. Again, chest imaging beyond 5 years is at the discretion of the physician and based upon individual patient risk factors [93].

9.9 Approach to the Patient with Localized RCC

Kidney cancer remains the most lethal of all urologic cancers with over 20 % of patients diagnosed with kidney cancer succumbing to the disease [3]. Despite a notable increase in early detection and extirpative surgery for localized kidney cancer, RCC-related mortality continues to rise [2, 6]. The implication is that while a fraction of RCC is aggressive and potentially lethal, a large proportion of early-stage RCC provides little, if any, impact on patient survival.

There are very limited level I data regarding optimal management of early-stage RCC. A recent meta-analysis of published data on the management of SRMs provides further confirmation that SRMS can be effectively managed with NSS, thermal ablative, or active surveillance [88]. Furthermore, a delay in surgical therapy for SRMs does not appear to affect cancer-specific survival [94]. This leads to an important question as to whether this level of aggressive therapy alters the natural course of SRMs.

Moreover, as oncologic data have demonstrated an equivalency of nephron-sparing surgery to RN, increased attention has focused on nephron preservation and the underutilization of NSS techniques. A recent examination of the National Cancer Database (NCDB) from 1993 to 2005 revealed that only 27.1 % of tumors less than 4.0 cm were being treated with NSS techniques [49]. At the beginning of this time period, a paltry 5.9 % of T1a lesions were being treated with NSS approaches. The SEER registry data shows similar trends. Examining the SEER data from 1999 to 2006 for over 18,000 lesions less than 4.0 cm, the rate of PN only increased from

20.0 to 40.0 % [43]. Finally, an analysis of over 66,000 patients from the Nationwide Inpatient Sample from 1988 to 2002 revealed a 7.5 % national rate of PN [95].

An important focus of modern-day oncologic practice is not solely on cancer-specific survival, but also on assessment of competing risks and their impact on clinical decision making. Considering the natural history of early-stage RCC, the benefit of surgical treatment depends in large part on an analysis of competing risks. In that respect, clinically localized RCC mimics early-stage prostate cancer in that it challenges the urologist to account for comorbidities that may contend with CSS. Recently published reports indicate that CCI scores are useful prognosticator of survival in patients with localized kidney tumors [25]. Surgical resection of SRMs with CCI scores greater than 2 appears to provide no survival advantage. This implies that the severity of comorbidities rather than the tumor itself dictates outcomes in early-stage RCC.

Using the SEER database, a first comprehensive nomogram estimating competing risks of death from localized RCC versus other cancer and non-cancer-related mortality came out in early 2010 [27]. This prediction model demonstrates that patients with localized node-negative kidney cancer have an excellent 5- (96 %) and 10-year (93 %) cancer-specific survival, while a significant 5- and 10-year overall risk of death from other cancers (7 %, 11 %) and non-cancer-related mortality (11 %, 22 %) exists. Furthermore, tumor size was a significant predictor of RCC-related death. Age, however, was a strong predictor of non-RCC-related death.

As surgical expertise in treatment of SRMs continues to evolve so does the concept of individualized patient treatment that integrates age and existing comorbidities. Although surgical treatment of SRMs is still heralded as the "gold standard," newly published AUA guidelines support active surveillance for appropriately selected patients with decreased life expectancy and extensive comorbidities [5]. Therefore, the use of objective tools, such as statistical models, nomograms, and nephrometry, for objectifying risk should become standard and not simply an option.

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Cytoreductive Nephrectomy

10

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Abbreviations

ccRCC	Clear cell renal cell carcinoma
CN	Cytoreductive nephrectomy
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
IL-2	Interleukin-2
MDACC	MD Anderson Cancer Center
mRCC	Metastatic renal cell carcinoma
MSKCC	Memorial Sloan Kettering Cancer Center
NCI	National Cancer Institute
OS	Overall survival
PFS	Progression-free survival
RCC	Renal cell carcinoma
SWOG	Southwest Oncology Group
UCLA	University of California Los Angeles

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_10

Take-Home Points (Highlights)

1. Cytoreductive nephrectomy (CN) is the current standard of care for *select* patients with metastatic clear cell renal cell carcinoma (mccRCC) *prior to planned immunotherapy.*
2. CN continues to have a significant role in the multidisciplinary care of select patients with mRCC treated with contemporary targeted therapies.
3. The role and timing of CN in the context of contemporary targeted treatments are undefined.
4. Judicious rather than ubiquitous use of CN is recommended by utilizing overall disease prognostic factors and factors predicting outcomes after CN.
5. The majority of patients with non-clear cell histology or sarcomatoid dedifferentiation do not appear to benefit from CN.
6. CN in an elderly population is associated with a much higher risk of morbidity and mortality.
7. Laparoscopic techniques for CN are safe and may have less morbidity when technically feasible in select patients.
8. Randomized trials integrating CN with contemporary agents are ongoing to define the role and timing of CN.

10.1 Introduction

Cytoreductive nephrectomy (CN) continues to be an integral part in the contemporary multidisciplinary treatment paradigm for patients with metastatic renal cell carcinoma (mRCC). Unlike many other cancers, removal of the primary tumor in mRCC has been shown to significantly increase overall survival (OS) when combined with postoperative cytokine therapy [1–3]. This was based on two randomized trials with a combined median increase in OS of 5.8 months. Since the FDA approval of the first systemic targeted therapy in 2005, CN has remained prevalent despite controversies regarding the optimal integration of surgery into the contemporary systemic targeted therapy paradigm. Two large phase III randomized trials are underway to assess the role and timing of CN in patients receiving the tyrosine kinase inhibitor, sunitinib malate [4, 5]. While awaiting the results of these trials, it is imperative for the treating physicians to understand the risks associated with CN and optimal patient selection for surgery. This chapter will highlight the historical evolution of CN in the treatment of metastatic RCC, review the data regarding optimal patient selection, highlight the risks of CN, and explore future methods on how to better integrate surgery into the treatment of patients with metastatic RCC.

10.2 Historical Perspective

Prior to the prospective randomized trials showing a survival advantage to CN, removal of the primary tumor was performed for three reasons: (1) palliation in patients with significant local symptoms from the primary tumor, (2) to induce spontaneous regression of metastatic sites, and (3) to improve response to endocrine or immune therapy.

Historically, surgical removal of the primary tumor in the setting of metastatic disease was performed for palliation of medically intractable symptoms attributed to the tumor [6, 7]. Refractory symptoms included gross hematuria, flank pain, bowel obstruction, high-output cardiac failure secondary to intratumoral arteriovenous fistulae, clot colic/urinary obstruction, and uncontrollable paraneoplastic syndromes [8]. The indication for a palliative nephrectomy was relatively rare and today is almost nonexistent with current medical, endovascular, and endourologic interventions (i.e., bisphosphonates, angioinfarction, ureteral stenting, etc.) [9–11].

In addition to the early use of CN in the palliative setting, surgical removal of the primary tumor in asymptomatic patients was performed in some centers on the basis of anecdotal reports of spontaneous regression of distant metastases subsequent to CN. Early hypotheses behind the spontaneous regression of mRCC were based on tumor-host interactions of the endocrine and immunologic systems [12, 13]. The incidence of spontaneous tumor regression was very rare (<0.8 %) with many of the reported cases

occurring in patients that had not received CN [14]. This phenomenon appears to be a reflection of the heterogeneous behavior of mRCC and the potential for misclassification of "metastatic disease" rather than a consequence of surgical intervention. When considering the operative mortality and significant morbidity, performing CN for the sole expectation of inducing spontaneous regression is not justified.

Many of the initial reports of endocrine and immunotherapies for mRCC suggested an improved response in patients after removal of the primary tumor [15–17]. The question of whether these findings were due to biases in patient selection would not be answered until nearly a decade later. The potential benefit of CN had to be balanced with the risk of early progression or morbidity from surgery, which would have precluded subsequent systemic therapy. In one of the early reports on interleukin-2 therapy (HD-IL-2), investigators at the NCI showed that 40 % of patients initially deemed eligible for systemic therapy would subsequently fail to receive IL-2 due to a combination of rapid disease progression or complications occurring after CN [16]. Although inherent biases in these reports limited the evaluation of CN on patient outcomes, these series provided a basis for two randomized clinical trials in the 1990s which would change the standard of care in mRCC.

10.3 Randomized Trials

In 2001, the pooled results of two randomized trials were published demonstrating a significant OS advantage in patients with mRCC who received CN prior to interferon alpha [2, 3]. The Southwest Oncology Group (SWOG) trial 8949 and the European Organization for the Research and Treatment of Cancer (EORTC) trial 30947 randomized patients with mRCC to either nephrectomy followed by interferon alpha or interferon alpha monotherapy. The eligibility criteria for both trials were the same: diagnosis of mRCC (spread beyond regional lymph nodes) with a resectable primary tumor in place, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, a serum creatinine <3.0 mg/dl, and a serum total bilirubin of less than three time the upper limit of normal. In a combined analysis of the two trials ($n=331$), the median survival for patients receiving interferon monotherapy was significantly lower than combined nephrectomy with subsequent interferon therapy, 7.8 months versus 13.6 months, respectively [3]. This 5.8 month difference in OS represents a 31 % decrease in the risk of death (Fig. 10.1). With the report of these two randomized trials, cytoreductive nephrectomy became the standard of care for many patients with synchronous mRCC prior to planned treatment with cytokine therapy.

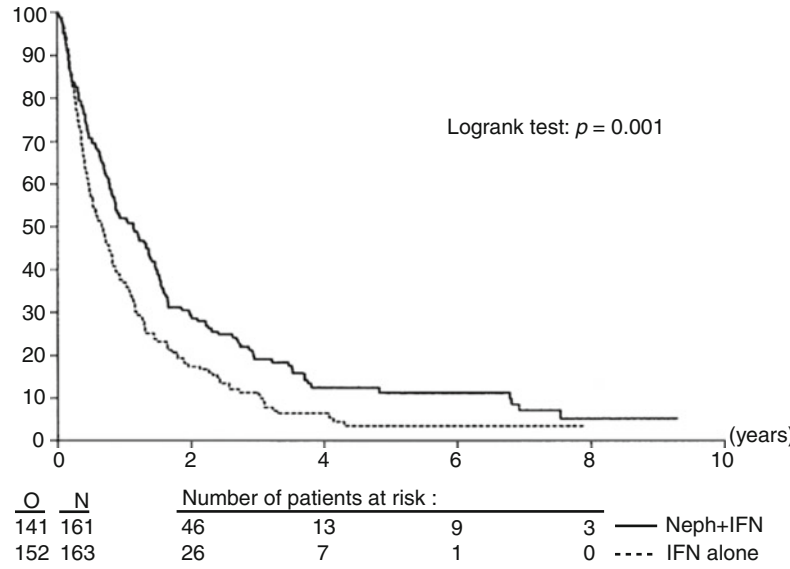

Fig. 10.1 Combined analysis of overall survival in the EORTC 30947 and SWOG 8949 trials. Median overall survival is 7.8 months in the observation group (O=interferon monotherapy) versus 13.6 months in the nephrectomy group (N = nephrectomy + interferon) (Adapted with permission from Flanigan et al. [3])

10.4 Proposed Mechanisms of Action

There are multiple hypotheses behind the survival advantage associated with CN prior to immunotherapy. With specific relevance to immunotherapy, large bulky primary tumors may act as an immunologic sink, and thus, removal of the primary tumor and bulky retroperitoneal lymph nodes may allow for an increased effectiveness of immunotherapy [16, 18, 19]. The primary tumor may also produce numerous growth and angiogenic factors which may contribute to the development and viability of distant metastatic disease (VEGF, TGF-ß1, PDGF, IL-8, IL-10, and FGF) [20–22]. A novel and interesting hypothesis reported by Gatenby et al. proposed that removal of the kidney and subsequent metabolic acidosis (rather than cytoreduction through removal of the primary tumor) was responsible for the increase in OS seen in the SWOG 8949 trial. The exact mechanism by which CN adds to OS is currently unknown.

10.5 Contemporary Therapies

CN in the era of molecular targeted agents has not been prospectively evaluated but has been generally accepted based on the earlier studies when performed prior to cytokine-based therapy [23, 24]. The current 2015 NCCN guidelines recommend CN in properly selected patients prior to *immunotherapy* [25]. With regard to patients prior to contemporary systemic targeted therapy, the NCCN guidelines cite retrospective series showing CN continues to play a role in patients treated with VEGF-targeted agents while anticipating the results from contemporary randomized trials [4, 5].

The overwhelming majority (90+%) of patients enrolled in clinical trials of targeted therapies have by default undergone prior CN. The one exception has been in the Global ARCC study of the mammalian target of rapamycin (mTOR) inhibitor, temsirolimus [26]. ARCC was a phase 3 randomized trial of interferon alpha, temsirolimus, or a lower dose combination of both agents in patients with poor risk mRCC of any histology. The primary tumor was not removed in 33 % of the patents and 20 % had non-clear cell histology. Temsirolimus improved OS among patients with mRCC and poor prognosis features. A subsequent subset analysis of this trial explored the influence of nephrectomy and histology on overall and PFS [27]. The improvement in PFS and OS in patients treated with temsirolimus was seen in both clear and non-clear cell histologies, while nephrectomy status did not impact the PFS or OS. Most of these patients had poor prognostic features and would not have been ideal candidates for CN [28].

Multiple retrospective analyses of patients treated with targeted therapy have provided conflicting data on the benefit of CN. In a multi-institutional analysis, Choueiri et al. reported on the outcomes of 314 patients receiving targeted therapy for mRCC [29]. Favorable risk and younger patients were more likely to undergo CN. After adjusting for significant differences in baseline prognostic factors, patients undergoing CN had a significantly improved OS (HR 0.44; CI 0.32–0.59, $p<0.01$). As would be expected, this survival advantage did not extend to patients classified as poor risk (similar to Global ARCC).

Heng et al. published a large multi-institutional data set assessing the benefit of CN from patients enrolled on the International Metastatic Renal Cell Carcinoma Database Consortium [30]. A total of 1,658 patients with a diagnosis of synchronous mRCC were included, with 982 of these patients undergoing CN and 676 not receiving surgery. As expected, baseline clinical prognostic factors varied between the groups with those undergoing CN having more favorable clinical features. After adjusting for the prognostic differences between the two groups, the authors reported results favoring CN with the median OS (20.6 vs. 9.5 months; $p<0.0001$; Fig. 10.2) and PFS (7.6 vs. 4.5 months; $p<0.001$). In this series, patients surviving less than 12 months did not appear to benefit from CN. This study, along with others, highlights the importance of selecting patients most likely to benefit from CN [31].

There are currently two phase III randomized trials attempting to provide insight into the role of CN in the era of targeted therapy and on the

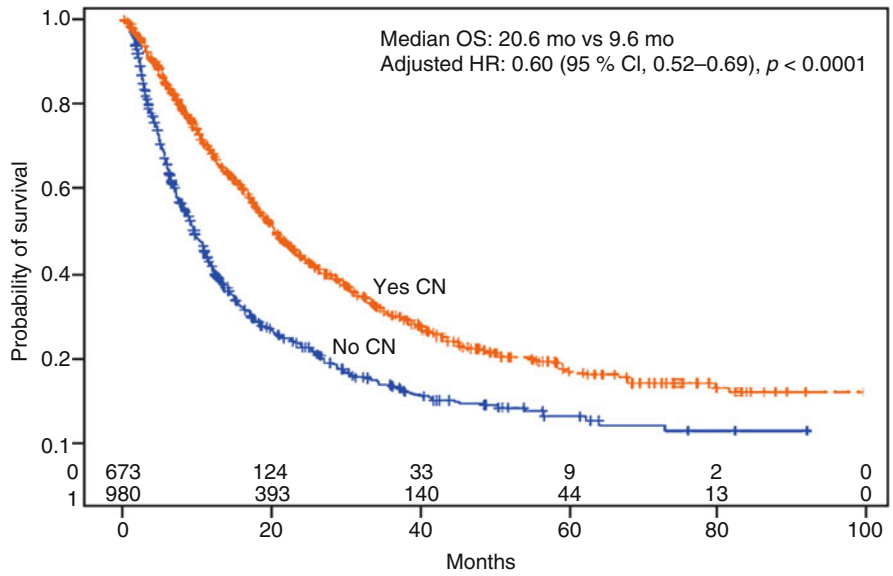

Fig. 10.2 Kaplan-Meier overall survival from the initiation of targeted therapy for 1,633 mRCC based on receiving a cytoreductive nephrectomy [30]

question of timing of nephrectomy with regard to systemic targeted therapies. The CARMENA trial is a randomized phase III trial comparing the first-line treatments of (1) CN followed by sunitinib to sunitinib monotherapy in clear cell RCC [4]. The anticipated enrollment is 576 patients with a primary end point of OS. This study will provide the only level I evidence assessing the role of CN in patients with mRCC treated with contemporary systemic targeted therapies. The second trial is being performed through the EORTC by randomizing patients to either (1) upfront CN followed by sunitinib versus (2) four 6-week cycles of sunitinib (4 + 2 schedule) followed by CN only in patients with non-progressive metastases [5]. The study is attempting to enroll 458 patients with the primary end point being PFS. Given the morbidity of CN and the considerable percentage of patients experiencing progression in the interval between surgery and the start of systemic therapy, this trial may provide evidence supporting the presurgical treatment of mRCC patients as a "litmus test" to further select candidates for CN. Completion of accrual and results from both of these trials are highly anticipated.

10.6 Patient Selection for CN

Based on the two randomized trials published in 2001, CN became the standard of care in patients with mRCC who are candidates for systemic immunotherapy. A very important caveat to the successful integration of surgery with systemic therapy is in defining the optimal patient selection criteria. CN can be associated with significant morbidity, which may preclude subsequent systemic therapy. In addition to complications and postoperative pain, some patient's disease will rapidly progress while recovering from surgery and they subsequently may be unsuitable to receive systemic therapy. Reports from the immunotherapy era showed significant variation in the percentage of patients who were unable to receive postoperative systemic therapy (range 5.6–77 %) because of complications of surgery or rapid disease progression (Table 10.1) [3, 16, 32–36].

10.6.1 Predictive Variables

In one of the initial studies from the National Cancer Institute, Walther et al. reported on a

Table 10.1 Cytoreductive series from the immunotherapy era

Study	Year	Number of patients	Institution	Morbidity	Operative mortality	% inability to receive systemic therapy
Walther et al.	1993	93	Single center	13 %	0 %	40 %
Rackley et al.	1994	37	Single center	16 %	2.7 %	22 %
Bennett et al.	1995	30	Single center	50 %	17 %	77 %
Franklin et al.	1996	63	Single center	12.7 %	0 %	12 %
Fallick et al.	1997	28	Single center	NR	3.6 %	7 %
Levy et al.	1998	66	Single center	35 %	3	18.1 %
Flannigan et al. (SWOG+EORTC)	2004	331	Multicenter	23.4 %	1.4 %	5.6 %

series of 93 patients undergoing CRN with planned postoperative interleukin-2 therapy [16]. Forty percent of patients were not able to receive postoperative systemic therapy, most commonly due to rapid progression of systemic disease. Preoperative clinical factors and laboratory values were assessed in an attempt to identify factors associated with failure to receive subsequent therapy. The only significant predictor of not receiving subsequent therapy was having a preoperative ECOG PS >1 ($p=0.047$).

In an attempt to mitigate the risks of CN, Fallick et al. used strict criteria to select patients for CN [35]. Patients being considered for CN had an ECOG PS of 0 or 1; predominant clear cell histology; greater than 75 % debulking of tumor burden technically feasible; absence of central nervous system, liver, or osseous metastases; and no major comorbid medical conditions. Over a 5-year time period, 85 patients with mRCC with their primary tumor in place were evaluated for CN. Patients in whom pretreatment biopsy revealed non-clear cell predominance were not considered for surgery. Only 33 % (28/85) met the eligibility criteria for CN. By utilizing these selection criteria, the operative outcomes and the ability to receive subsequent systemic therapy (93 %) were improved over prior series. Investigators at the Cleveland Clinic performed an independent analysis of metastatic burden in 46 patients undergoing CN [37]. In this contemporary series of patients undergoing CN, fractional percentage of tumor volume (FPTV) was shown to be associated with survival. In this cohort of patients treated only with targeted therapies, FPTV removed (<90 % versus ≥90 %) and preoperative corrected calcium were independent predictors of progression-free survival (PFS) (Fig. 10.3) [37].

Published selection criteria for the two randomized trials were not as strict as those by Fallick et al. Eligibility for the SWOG 8949 and EORTC 30947 was identical [1–3]. All patients had a prerandomization biopsy, adequate liver function (bilirubin <3× ULN), adequate renal function (Cr <3.0 mg/dl), ECOG PS of 0 or 1, and no prior malignancy within 5 years. Only 5.6 % of patients were unable to receive postoperative interferon alpha. In a later analysis of the SWOG 8949 data, Lara et al. analyzed predictive variables for OS after CN (Table 10.2) [38]. On multivariate analysis, patients with early progression (<90 days) and patients with an ECOG PS of 1 (versus 0) had significantly worse OS. Of course, early progression is not a preoperative variable, but perhaps identification of patients showing earlier signs of progression (SURTIME) would be desirable and aid in selection of patients for aggressive multimodal treatment through the use of CN.

In 2006, a multidisciplinary panel used available data and the RAND/UCLA Appropriateness Method to develop recommendations regarding optimal patient selection [39]. Patients were classified as "good risk" surgical patients if the Eastern Cooperative Oncology Group (ECOG) performance status was 0 or 1 and major comorbid conditions were absent. Metastatic burden was classified as lung metastases only, limited metastases (low-volume lung or bone disease), or extensive burden (lung and bone metastases or any liver or CNS involvement). Symptoms were

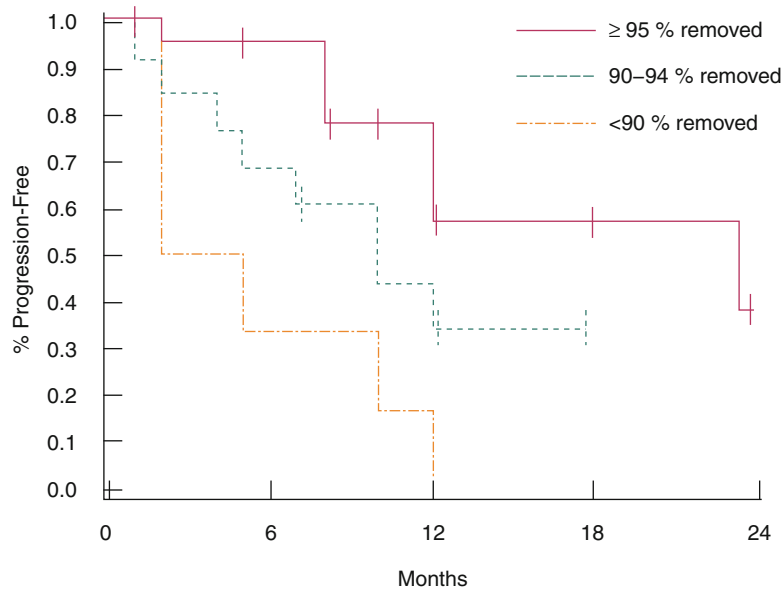

Fig. 10.3 Progression-free survival by fractional percentage tumor volume (Adapted with permission from Barbastefano et al. [37])

Table 10.2 Multivariate analysis of predictors of overall survival after 90 days

Clinical variable	HR (95 % CI)	*P* value
Progression by 90 days	2.10 (1.50–2.92)	<0.0001
ECOG PS (1 versus 0)	1.70 (1.26–2.31)	0.0006
Lung metastasis (yes vs. no)	0.81 (0.59–1.11)	0.019
Alkaline phosphatase[a]	1.24 (0.92–1.68)	0.26
Hemoglobin[a]	0.84 (0.62–1.14)	0.26

[a]Above versus below the median

defined in relation to the primary tumor. The panel recommendations were as follows: for good surgical risk patients with planned *postoperative immunotherapy*, nephrectomy was rated appropriate in patients who had limited metastatic burden regardless of symptoms and in symptomatic patients regardless of metastatic burden. With regard to planned *targeted therapy*, the panel recommended only patients with the most favorable combination of surgical risk, metastatic burden, and symptoms undergo CN. The panel highlighted the limitations in defining the role of CN in patients for whom systemic targeted therapy is planned.

In addition to selection criteria established from single center retrospective series and the two randomized trials, many authors incorporate prognostic factors for OS when deciding appropriateness of CN. Whether these overall prognostic factors can be used to predict for early progression and thus be used as selection criteria for CN is unknown. Although the MSKCC risk stratification is one of the most widely accepted and validated set of prognostic factors in mRCC, this stratification system was not intended for use as a selection criterion for performing CN, but rather was established to provide prognostication for patients undergoing systemic therapy alone or *after* CN [40–42]. Utilizing these prognostic variables, patients are further categorized into favorable risk (0 risk factors), intermediate risk (1–2 risk factors), or poor risk (≥3 risk factors). A poor prognostic variable in the initial report was absence of a nephrectomy (presence of the primary tumor) [42]. Due to the rapid adoption of CN after the SWOG 8949 and EORTC 30947 publications, the subsequent MSKCC criteria replaced this variable with "time from diagnosis to treatment of less than 12 months" (Table 10.3) [41]. The original as well as subsequent modified risk stratification systems have also been shown to be useful for prognostication in contemporary cohorts of mRCC patients receiving targeted therapy [43–45].

Several of these validated prognostic factors for OS after systemic therapy were either

Table 10.3 Poor prognostic factors for overall survival in patients with metastatic renal cell carcinoma

Study	Motzer et al.	Motzer et al.	Mekhail et al.	Motzer et al.	Heng et al.	Patil et al.
Publication year	1999	2002	2005	2009	2009	2011
Therapy	Multiple	Interferon alpha	Interferon alpha or cytotoxics	Interferon alpha or sunitinib	Sunitinib, sorafenib, bevacizumab	Interferon alpha or sunitinib
Number (n)	670	463	308	750	645	750
Risk criteria						
Favorable	0	0	0–1[a]	0	0	n/a
Intermediate	1–2	1–2	2	1–2	1–2	n/a
Poor	>2	>2	>2	>2	>2	n/a
Prognostic factors	KPS <80	KPS <80	–	ECOG PS >1	KPS <80	ECOG PS >1
	Low HGB	Low HGB	Low HGB	Low HGB	Low HGB	Low HGB
	Corrected calcium ≥10 mg/dl	Corrected calcium ≥10 mg/dl	Corrected calcium ≥10 mg/dl	Corrected calcium ≥10 mg/dl	Corrected calcium ≥10 mg/dl	Corrected calcium ≥10 mg/dl
	LDH >1.5× ULN	LDH >1.5× ULN	LDH >1.5× ULN	LDH >1.5× ULN	–	LDH >1.5× ULN
	Absence of prior nephrectomy	Time from diagnosis to systemic therapy <12 months	Time from diagnosis to systemic therapy <12 months	Time from diagnosis to systemic therapy <12 months	Time from diagnosis to systemic therapy <12 months	Time from diagnosis to systemic therapy <12 months
			>1 organ site with metastases	>1 organ site with metastases	Neutrophils > ULN	Osseous metastases
			Prior radiotherapy	Treatment with interferon (versus sunitinib)	Platelets > ULN	
			Retroperitoneal lymph node disease			

KPS Karnofsky Performance Status
ECOG PS Eastern Cooperative Oncology Group Performance Status
HGB Hemoglobin
LDH Lactate dehydrogenase
ULN Upper limit of normal
[a]Mekhail et al. is unique in the number of factors to define risk groups

previously incorporated into patient selection criteria for CN or have been subsequently analyzed. Given the significant morbidity of CN, the indiscriminant use of CN is not advisable [22]. Kutikov et al. reported on the outcomes of 141 patients after CN treated between 1990 and 2008 [46]. Of those not receiving subsequent systemic therapy (30.5 %: 43/141), the most common reason was rapid disease progression (30.2 %). Patients not receiving systemic therapy had a trend toward lower survival although this was not statistically significant ($p=0.16$). The risk of death after surgery correlated with the number of metastatic sites ($p=0.012$), symptoms at presentation ($p=0.001$), poor performance status ($p=0.001$), high tumor grade ($p=0.006$), and the presence of sarcomatoid features ($p<0.024$).

Although there are significant practice variations among high-volume centers, the selection of patients for CN is based on a combination of prognostic factors for OS and predictors of surgical outcome after CN. In one of the largest series of its kind, Culp et al. attempted to identify preoperative clinical variables in a cohort of 566 patients undergoing CN and 115 receiving systemic therapy alone at the MD Anderson Cancer Center over a 15-year period (1991–2007) [31]. An extensive list of preoperative variables was analyzed which resulted in the identification of seven preoperative variables found to be significant negative predictors of overall survival (Table 10.4). The number of preoperative risk factors was correlated with OS and was inversely proportional to the median survival of patients who underwent CN. *Patients who underwent CN with >3 preoperative risk factors did not appear to benefit from CN when compared to patients undergoing medical therapy alone* (Fig. 10.4). Sarcomatoid dedifferentiation and Fuhrman grade 3 or 4 were also significant factors for OS, but in most cases these were not available preoperatively and thus were not included in the analysis of preoperative factors.

Table 10.4 Negative preoperative prognostic factors for overall survival after cytoreductive nephrectomy

Preoperative variable	HR (95 % CI)	*P* value
Albumin < LLN	1.59 (1.21–2.10)	0.001
LDH > ULN	1.66 (1.26–2.18)	<0.001
cT3 or cT4	1.37 (1.01–1.87)	0.019
	2.05 (1.13–3.72)	0.041
Symptoms from metastatic site	1.35 (1.03–1.75)	0.028
Liver metastases	1.47 (1.02–2.13)	0.039
Retroperitoneal lymphadenopathy	1.29 (1.01–1.63)	0.04
Supradiaphragmatic lymphadenopathy	1.48 (1.18–1.86)	0.001

LLN Lower limit of normal
ULN Upper limit of normal
LDH Lactate dehydrogenase

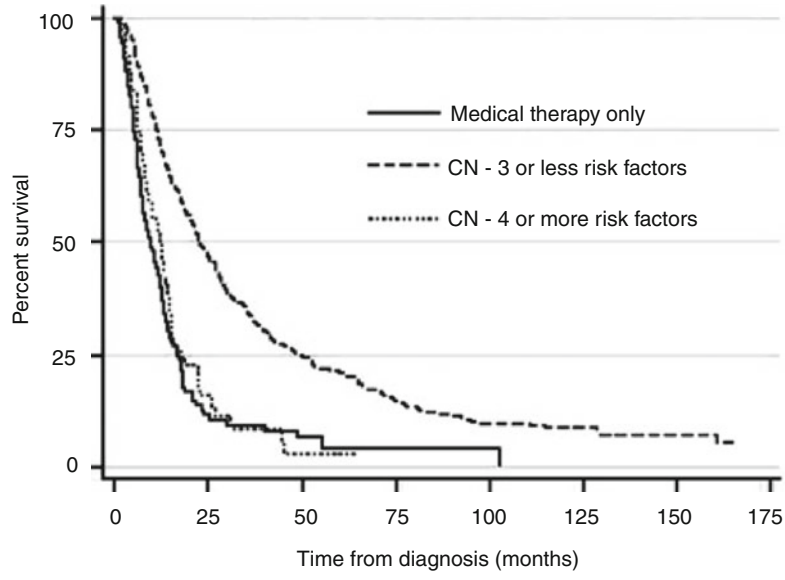

Fig. 10.4 Kaplan-Meier analysis of overall survival for patients with metastatic renal cell carcinoma (mRCC) who underwent cytoreductive nephrectomy based on the number of preoperative risk factors (see Table 10.4). The solid line represents mRCC patients treated with medical therapy alone (Adapted with permission from Culp et al. [31])

10.6.2 Elderly

Aggressive surgical resection in elderly (age ≥75 years) patients with mRCC should be performed only in highly selected candidates. Kader et al. assessed the outcomes of 24 elderly patients undergoing CN at the MD Anderson Cancer Center (MDACC) and compared them to another 380 patients (<75) undergoing CN [47]. Despite the preoperative prognostic factors being similar between groups, the peri-operative death rate was significantly higher in the elderly patients (21 versus 1.1 %). Although the two groups had a similar median OS, the authors suggested CN should be used judiciously in highly motivated and carefully selected elderly patients.

10.6.3 Non-clear Cell Histology

The data regarding cytoreductive nephrectomy in patients with non-clear cell RCC is scarce and conflicting. While all non-clear cell histologies portend a relatively poor prognosis when metastatic, patients with M1 papillary disease appear to have a worse OS than those with chromophobe histology (median 5.5 versus 29 months) [48]. Interestingly, patients with regional nodal metastases from papillary RCC in the absence of detectable metastatic disease (N1M0) have a relatively indolent clinical course, which authors have suggested may be due to a biologic difference in vascular versus lymphatic predominant papillary RCC [49, 50]. Currently, efficacious systemic therapies for metastatic non-clear cell RCC are lacking, and many clinicians consider non-clear cell histology or sarcomatoid dedifferentiation a contraindication to CN [51].

Kassouf et al. examined the outcomes of 606 patients undergoing CN from 1991 to 2006. Of these, 92 patients had non-clear cell RCC [52]. On multivariate analysis, DSS in patients with non-clear cell RCC was significantly worse than patients with clear cell RCC (9.7 vs. 20.3 months, $p=0.003$). The presence of sarcomatoid features was also a poor prognostic variable in both clear (HR 1.8: CI 1.3–2.4, $p=0.001$) and non-clear RCC (HR 2.8: CI 1.5–5.2, $p=0.002$). Although sarcomatoid dedifferentiation is not a histologic subtype, its presence is almost universally associated with a very poor prognosis. In an analysis of 417 CN cases at UCLA, Shuch et al. identified 62 tumors with any percentage of sarcomatoid dedifferentiation [53]. The median survival of patients with sarcomatoid was 4.9 vs. 17.7 months in patients without sarcomatoid components ($p<0.001$). The authors concluded CN was not beneficial in patients with sarcomatoid components.

In an attempt to assess the diagnostic sensitivity of percutaneous biopsy in the preoperative identification of sarcomatoid feature, Abel et al. identified 166 patients who had received percutaneous biopsy prior to CN at the MDACC [54]. At the time of nephrectomy, 20.5 % (34/166) of specimens contained sarcomatoid components. Only four (11.8 %) were identified preoperatively by biopsy. The median survival of patients with sarcomatoid components was 4.9 versus 17.7 months in those without sarcomatoid features. Only 41.9 % of patients with sarcomatoid features proceeded to receive systemic therapy. Unfortunately, the utility of a percutaneous biopsy to preoperatively identify sarcomatoid components is poor. Despite the sparse data on patients with non-clear cell histology, CRN for patients with non-clear cell histology is usually only considered for patients with exquisitely optimal prognostic factors (other than histology).

10.7 Surgical Technique

The predominant surgical technique in published series and trials of CN has been an open surgical approach. The earliest series of laparoscopic CN was published by investigators at the NIH [55]. When technically feasible, the laparoscopic approach potentially offers a shorted hospital stay, reduced blood loss, earlier time to systemic therapy, and less postoperative pain [55–58]. Rabets et al. found a shorter time to systemic therapy with the laparoscopic approach (36 versus 61 days), while a report by Matin et al. showed reduced blood loss and length of hospital stay, yet failed to show a reduced time to systemic therapy

[56, 58]. Finelli et al. reported on a series of 22 patients undergoing laparoscopic CN at the Cleveland Clinic [56]. The authors concluded laparoscopic CN is safe in selected patients with tumors ≤15 cm and no evidence of adjacent organ invasion (cT4) or inferior vena caval thrombi while cautioning that significant perihilar adenopathy or an abundance of parasitic vessels may increase the complexity of the surgery. With increasing expertise in minimally invasive surgical techniques, there is likely to be increasing utilization of these approaches in performing CN.

10.7.1 Lymph Node Dissection (LND)

The role of lymph node dissection in the setting of CN is controversial. The presence of concomitant nodal and distant metastatic disease was shown to be a significant predictor of OS in patients undergoing CN [19, 31, 59]. In a series of 1,153 metastatic patients undergoing CN, Lughezzani showed the cancer-specific mortality rates of patients with pNxM1, pN0M1, and pN+M0 were significantly different (66 %, 65 %, and 86 %, $p<0.001$ respectively) [59]. Concordant with the findings of Culp et al. [31], lymph node status was an informative predictor of outcomes after CN, and the authors suggested inclusion of this variable in future prognostic models.

The therapeutic role of lymph node dissection in the setting of CN has been evaluated in several retrospective series from the cytokine era. The National Cancer Institute evaluated a cohort of 154 patients that underwent CN prior to systemic IL-2 [19]. The authors compared 82 clinically node-negative patients (cN0M1) with 72 patients with clinically positive lymph nodes (cN+M1). The median survival for clinically node-negative and node-positive patients was 14.7 and 8.5 months, respectively ($p=0.0004$). Interestingly, no statistically significant difference in survival was noted between patients with clinical N0 disease and those with retroperitoneal lymphadenopathy (LAD) completely resected (cN+ made NED by resection) (14.7 vs. 8.6, $p=0.07$). Although this statistical difference suggested a therapeutic effect of LND, the study was underpowered to make any conclusive statements. Patients whose nodes were incompletely resected still maintained an overall survival of 8.5 months (comparable to those with cN+ disease), while those with unresectable LN disease had a dismal 3.3-month survival. However, this survival difference did not appear to be secondary to improved response rates to IL-2 therapy. As resection did not change the response to systemic therapy, whether a more complete cytoreduction with resection of LAD changes the natural history of disease is unknown.

Pantuck et al. assessed the impact of lymph node-positive disease on a large cohort of metastatic patients (322 M1) treated with CN at UCLA [18]. In this study, 236 patients with clinical N0M1 RCC were compared to 86 patients with clinical N+M1 disease. Both groups received postoperative immunotherapy at the same rate (65 %). Similar to previous reports, the median survival was 20.4 months for N0M1 versus 10.5 months in patients with N+M1. In a separate analysis including patients with N+M0 disease, the authors found no perceived survival benefit to IL-2 in those patients with unresected clinically positive lymph nodes [60]. Patients with clinically positive lymph nodes undergoing nephrectomy with synchronous LND ($n=129$) had a significant survival advantage (5 month improvement) over those patients with clinically positive nodes left in situ ($n=17$). The analysis included patients with N+M0 disease ($n=43$), and it is unclear whether this perceived survival advantage is due to the heterogeneous population studied. Although no strong conclusions can be made in patients with M1 disease, these retrospective series suggest the natural history of disease in patients with mRCC treated with immunotherapy may be altered by LND.

10.7.2 Partial Nephrectomy (Nephron Sparing)

In a very select group of patients, partial nephrectomy (PN) does not appear to compromise oncologic outcomes and may have a role in cytoreduction [61–63]. The optimal patients

considered for PN would have low-volume metastatic disease especially considering the correlation between percentage cytoreduction and survival after CN [35, 37]. Although patients with significant comorbidities or chronic renal insufficiency (Cr >3) have been excluded from most series of CN, some authors have proposed partial CN in highly selected patients with a solitary kidney, renal insufficiency, or bilateral tumors [64]. Authors from the Mayo Clinic reported on the outcomes of 16 patients after partial CN. Indications for partial CN were the presence of mRCC and a solitary kidney (75 %), bilateral disease (19 %), or elective (6 %). While cancer-specific survival rates were comparable to patients undergoing removal of the entire kidney, patients undergoing partial CN for a solitary kidney indication had higher postoperative rates of chronic renal insufficiency (25 %), proteinuria (25 %), and requirement of dialysis (17 %). In two larger series without data regarding postoperative complications, a significant difference in cancer-specific survival was not appreciated [62, 63]. The authors of both these series concluded partial CN does not appear to undermine survival if performed in highly selected cases.

10.8 Current Controversies and Future Directions

Newer targeted therapies are better at downsizing the primary tumor than immunotherapy, but reductions in size (diameter) are generally less than 30 % (RECIST stable disease) [65]. Several centers have reported small series of patients treated with targeted agents in the presurgical setting [66–69]. The first trial to evaluate the safety of presurgical targeted therapy was reported by Jonasch et al. [66]. In this phase 2 study, 50 patients with surgically resectable metastatic clear cell RCC were treated with bevacizumab or bevacizumab with erlotinib for 8 weeks. At 8 weeks, all patients were restaged and those patients with adequate performance status and without progressive disease received CN. Clinical outcomes were comparable to the use of targeted agents in the postsurgical setting, but delayed wound healing resulted in postoperative treatment delay in 10 %. Of the 50 patients on study, 18 % (8) did not receive CN. This was due to progressive disease (12 %; 6/50), coming off study due to drug side effects ($n=1$), or due to death unrelated to the study ($n=1$; motor vehicle accident). This study provided the initial data regarding the safety of integrating surgery with systemic targeted therapy. Whether the 12 % with early progressive disease were spared an unnecessary procedure or should be regarded as a missed opportunity for therapeutic intervention is only speculative.

Authors at the MD Anderson Cancer Center reported the results of a comparative retrospective study assessing safety of surgery in patients who received presurgical systemic therapy prior to CRN ($n=70$) and those who underwent immediate CN ($n=103$). Presurgical systemic therapy was not associated with an increased overall or severe (Clavien ≥ 3) complication rate. There was an increased rate of local wound complications in the presurgical group but overall clinical significance of this likely minimal. The results of the EORTC trial (SURTIME) assessing the timing of surgery will provide more substantive data regarding this question.

Conclusions

In good and intermediate risk patients with metastatic clear cell RCC, cytoreductive nephrectomy is the standard of care prior to planned immunotherapy (HD-IL-2). It is imperative that the treating physician understands the significant risks associated with CN and utilizes available prognostic factors to judiciously select patients for this potentially morbid surgical resection. When planning on systemic treatment with contemporary targeted agents, the use of cytoreductive nephrectomy is supported by large retrospective series but should be used in selected patients without significant poor prognostic factors. The results of two European trials will likely define both the role and timing of CN in patients with metastatic RCC being treated with systemic targeted agents.

Clinical Vignette

A 49-year-old man is found to have a 12 cm left renal mass with level 2 inferior vena caval thrombus, multiple 1–2 cm bilateral pulmonary metastases, and a left supraclavicular 3 cm lymph node. His only complaint is abdominal fullness and he has an ECOG performance status of 0. Percutaneous biopsy of the supraclavicular lymph node reveals high-grade clear cell renal cell carcinoma (RCC). Laboratory values are normal and no other sites of disease are detected. The patient and his multidisciplinary team discuss treatment options and available clinical trials. The patient undergoes left open cytoreductive nephrectomy revealing margin negative pT3b clear cell RCC without sarcomatoid components. Patient recovers from surgery without handicap and elects to receive high-dose interleukin-2 therapy. He has progression of his pulmonary nodules after two cycles of HD-IL-2 and subsequently receives sunitinib.

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Metastasectomy

11

Saeed Dabestani and Axel Bex

Contents

Key Points

- Retrospective data suggest that complete resection of solitary or oligometastasis at one organ site after a long disease-free interval is associated with a survival benefit. No randomized prospective trials have been performed and retrospective data are biased by variations in metastatic burden, performance status, and indications for metastasectomy. Nevertheless, a robust review of the evidence base, using rigorous methodology, consistently demonstrated survival benefits of local therapy for RCC metastases.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_11

- It is unclear if the prolonged survival observed in some individuals is due to the complete resection of metastatic disease or a consequence of a selection bias in which those with favorable prognostic factors have a higher chance to proceed to metastasectomy (Table 11.1).
- The lungs are the most frequent metastatic site in RCC and complete resection of fewer than seven pulmonary metastases has been associated with a 5-year survival rate of 37–54 %. Unilateral lung involvement, the absence of lymph node metastases, and smaller size are additional site-specific favorable factors.
- Liver metastasis has a poor prognosis. However, if complete resection can be achieved for solitary lesions, 5-year survival rates of 62 % have been reported. Hepatic metastasectomy is associated with significant morbidity and mortality and it is unclear if surgery is superior to ablative percutaneous techniques.
- Resection of bone metastasis is mainly performed for palliative reasons, but metastasectomy of metachronous and in particular appendicular solitary bone lesions may result in 5-year survival rates of 75 %. Contrary to symptomatic bone metastases where surgery is superior to radiotherapy, the best approach for asymptomatic solitary bone lesions is unclear. If surgery is selected, wide excision with durable fixation or reconstruction is preferable.
- Stereotactic radiosurgery for brain metastasis yields median survival of 24 months in patients with RTOG RPA prognostic class I. Craniotomy may be preferable in lesions >2–3 cm, rapid onset of symptoms, and lesions with midline shift. WBRT is only adequate for patients with poor performance.
- Since synchronous solitary adrenal metastases are often resected at the time of nephrectomy, little is known about the management of isolated metachronous ipsi- and contralateral adrenal lesions. Cases are often reported in series of local recurrences. Survival of up to 70 months has been reported after metastasectomy and a long metachronous interval.
- Isolated lymph node metastases without further systemic disease are rare. However, their removal may be potentially curative. Synchronous regional lymph node metastases are often resected at nephrectomy. Resection of metachronous isolated lymph node metastases is associated with long-term survival.
- Complete metastasectomy of solitary lesions in the pancreas, thyroid, and other less frequently involved sites results in 5-year survival rates comparable to those observed after pulmonary metastasectomy. Careful selection should be made according to the general clinical factors associated with a favorable outcome (Table 11.1).
- Repeat complete metastasectomy and complete resection of multiple metastatic sites are associated with long-term survival and a 50 % decrease in the risk of death. Careful selection should be made according to the general clinical factors associated with a favorable outcome (Table 11.1).
- Integration of targeted therapy with surgery may lead to more candidates for metastasectomy. Multiple case reports and series report benefits and prospective trials are ongoing.

Table 11.1 Clinical factors associated with a favorable outcome after metastasectomy. General and additional reported site-specific factors for the lungs, bone, and brain

General	The lungs	The bone	The brain
Solitary or oligometastatic lesions Metachronous metastasis and long disease-free interval of >2 years Complete resection Single organ site Good performance status (Karnofsky, ECOG, WHO) MSKCC good- and intermediate-risk Absence of sarcomatoid features Absence of lymph node metastases	<7 metastases Absence of mediastinal lymph node metastases Metastases <4 cm Unilateral lung involvement	Appendicular metastases Wide excision Clear cell subtype	RPA class I Karnofsky PS >70 % Age <65 years Absence of extracranial metastases

11.1 Introduction

Renal cell carcinoma (RCC) accounts for approximately 3 % of adult malignancies and 95 % of renal neoplasms [53]. In the European Union, there were approximately 85 new cases and 35 deaths per 100,000 in 2012 [31]. The figures are similar for the United States with approximately 64,000 new cases in 2014 [125]. Metastatic RCC is present in up to 30 % of patients at diagnosis with multiple sites affected in 95 % [32, 114]. An additional 40 % of those undergoing surgery for localized RCC will develop metastases later. Therefore, approximately 30,000 patients a year have metastatic disease in the European Union alone, of whom an estimated 7,000 demonstrate non-clear cell histology. Data from the Nationwide Inpatient Sample show a preference for certain sites with the lungs involved in 45.2 %, followed by skeletal metastases in 29.5 %, lymph nodes in 21.8 %, liver metastasis in 20.3 %, and brain in 8.1 % [15]. Other locations have been described but at a lower frequency. Despite the introduction of targeted agents, treatment of metastatic RCC presents a therapeutic challenge. Although objective responses following targeted therapy are observed in 40–30 % of patients, complete responses occur in only 1–3 % [45, 87, 88]. Moreover, it has become evident that despite the most effective drugs in first-line treatment, median overall survival is only marginally longer than 2 years, which may be extended to 40 months in selected patients with adequate sequential therapy [30]. Therefore, together with the occasional durable responses achieved with high-dose interleukin-2, surgical resection of all lesions, when technically feasible, provides the only potentially curative treatment. However, only a minority of patients with metastatic RCC are candidates for metastasectomy. No reliable data exist on the percentage of patients with metastatic RCC who will be eligible for metastasectomy. A population-based analysis revealed that up to 65 % of patients with metastatic RCC have a single disease site but most of them are either not solitary or not accessible for surgery [15]. It has been estimated that only 25 % of patients with metachronous metastases are suitable candidates for resection of metastatic disease [2, 29]. Regarding synchronous metastatic disease, this proportion may be much lower. A Scandinavian whole nation study on prevalence and potential resectability identified 154 patients (16.9 %) with synchronous lung metastases in whom the proportion of metastasectomy was evaluated [98]. Eventually, only 11 patients had single lesions deemed eligible for metastasectomy which was performed in only one patient. Additionally, proper patient selection for this approach is difficult due to the heterogeneous biology of

metastatic RCC. Metastasis may present at diagnosis or within a year after nephrectomy with rapid progression of disease, whereas in other individuals, disease-free intervals of more than 20 years have been observed followed by slow growth pattern of the metastatic lesions. In few cases spontaneous regression of metastases has been documented, which has been ascribed to the presence of effective immune surveillance [78, 147]. In summary, there is considerable uncertainty regarding the best approach to metastasectomy. The major reason is a complete lack of randomized studies in this setting. A recent systematic review addressed the question whether local therapy for RCC metastases is beneficial and what the best options are [19]. Conducted in accordance with Cochrane Review methodology, including all types of comparative studies on local treatment of metastases from RCC in any organ, 2,235 studies were identified, of which 16 studies reporting on a total of 2,350 patients were eligible for inclusion. All studies were retrospective comparative studies with small patient numbers. The results revealed a benefit for complete metastasectomy when compared to either incomplete or no metastasectomy for metastases to various organs in terms of survival and symptom control, such as pain relief in bone metastases. However, the overall extensive risks of bias across all studies resulted in a significant risk of confounding. Due to the relatively poor quality of the few comparative studies, the evidence retrieved in the review was associated with large uncertainty, and no general recommendations were made. Ultimately, proper selection of patients for metastasectomy is of paramount importance. Surgical resection alone or in combination with targeted agents may result in clinical efficacy that is superior to systemic therapy alone. Currently, management of metastatic disease is depending on a number of clinical factors such as performance status, the length of the disease-free interval, the presence of synchronous or metachronous metastases, as well as the number and location of sites involved [73]. One of the most commonly used prognostic models, the Memorial Sloan Kettering Cancer Center (MSKCC) risk-score model, has been established from a database of 670 patients treated with cytokines. A previously validated risk score based on Karnofsky performance status, interval from nephrectomy, and serum hemoglobin, calcium, and lactate dehydrogenase was used to categorize patients as being favorable, intermediate, or poor risk [89]. Metastasectomy is associated with survival and clinical benefit across these various risk groups [28, 29]. A retrospective analysis was performed in 129 patients with localized RCC treated with partial or radical nephrectomy who were subsequently diagnosed with disease recurrence. In the favorable-risk group, metastasectomy improved 5-year survival from 36 to 71 %. In the intermediate-risk group, 5-year survival was 38 % after metastasectomy as opposed to 0 % in the same risk group without metastasectomy or the poor prognosis group. Even after adjusting for risk score in a multivariate analysis, patients who did not undergo metastasectomy had a 2.7-fold increased risk of death. A previous cohort from the same institution included 118 patients who had a median survival time of 21 months from the time of recurrence [29]. Overall survival was strongly associated with risk group category ($p < 0.0001$). Median survival time and 2-year survival rates for low-risk, intermediate-risk, and high-risk patients were 76, 25, and 6 months and 88 % (95 % CI, 77 to 99 %), 51 % (95 % CI, 37 to 65 %), and 11 % (95 % CI, 0 to 24 %), respectively, suggesting that only patients with favorable- or intermediate-risk features are candidates for metastasectomy. Despite the introduction of targeted therapy, the MSKCC risk score remains a valid tool among other similar risk scores to identify potential candidates for metastasectomy [46, 104].

11.2 History of Metastasectomy and Evolvement of General Prognostic Factors

Before the advent of effective systemic therapy, patients with untreated metastatic RCC had a median overall survival of 10 months, with a 5-year survival rate of less than 10 %. After the introduction of cytokine therapy, overall survival

rates were only marginally improved. Surgery was the only chance for cure. Therefore, most of the literature on metastasectomy dates back to the 1960s and 1970s of the last century, when it became evident that patients with a solitary resectable metastasis or multiple metastases restricted to one resectable organ site may have a survival benefit. In 1939 a report was published on a patient who survived 23 years following the resection of pulmonary metastases [11]. One of the first series describing metastasectomy in 41 patients with solitary lesions in the lungs, pleura, central nervous system, and abdomen dates from 1978, an era devoid of effective systemic therapy. In patients in whom complete surgical resection was possible, the median disease-specific survival was 27 months with 59 % of the patients alive at 3 years [23]. Several authors reported a 3-year and 5-year survival after resection of a solitary lesion of 45 % and 29–34 %, respectively [85, 127, 140]. Others observed a significant difference in survival in patients with metachronous and synchronous metastases [97, 110, 141]. In 179 patients the 5-year survival rate after resection of solitary lesions at various sites was 22 % for synchronous versus 39 % for metachronous metastases [133]. In addition, multiple clinical trials involving cytokine therapy revealed a strong association between clinical outcome and metastatic sites [41, 136]. These findings were supported by a series including 101 patients who underwent resection of a total of 152 metastatic lesions at different organ sites [145]. The median survival was 28 months for the entire series. Survival was improved after resection of lung metastases compared to other tumor locations ($p=0.0006$) and for patients that were clinically tumor-free after metastasectomy ($p=0.0230$). Additional immuno- or radiotherapy did not independently influence survival. Again, time interval between primary tumor resection and metastasectomy correlated positively with survival: a tumor-free interval of more than 2 years between primary tumor and metastasis was accompanied by a longer disease-specific survival after metastasectomy. Patients with bone and liver metastasis had a worse outcome than those with pulmonary lesions [41, 145]. Five-year survival rates for solitary metastases were 56 % for lungs, 28 % skin, 20 % visceral organ, 18 % peripheral bone, 13 % brain, and 9 % axial bone metastases [133]. In an attempt to define selection criteria for patients with solitary metastases, 278 patients with recurrent RCC were retrospectively analyzed [64]. The 5-year overall survival rate for 141 patients who underwent complete metastasectomy for their first recurrence, 70 patients who underwent incomplete metastasectomy, and 67 patients who were treated nonsurgically was 44 %, 14.5 %, and 11 %, respectively. Five-year overall survival rate was 55 % with a disease-free interval greater than 12 months versus 9 % with 12 months or less ($p<0.0001$), 54 % for solitary versus 29 % for multiple sites of metastases ($p<0.001$), and 49 % for age younger than 60 years versus 35 % if older ($p<0.05$). Among 94 patients with a solitary metastasis, the 5-year overall survival rate was 54 % for the lungs. Factors associated with a favorable outcome by multivariate analysis included a solitary site and single metastasis, complete resection of first metastasis, a long disease-free interval, and a metachronous presentation with recurrence. Since then, multiple retrospective series have been published that support these favorable factors [5, 41, 115] (Table 11.1). In particular complete metastasectomy is a cross-cultural favorable prognostic factor. In a series of patients from Japan who had nephrectomy and metastasectomy, survival was approximately twice as long as that of previous studies without metastasectomy [94]. In a recent large multicenter analysis from Japan, incomplete resection, elevated C-reactive protein, brain metastases, and high nuclear grade were confirmed as poor prognostic factors [93]. A caveat of the retrospective series remains the inherent bias of comparing patients with solitary and oligometastatic disease and a prolonged metachronous interval to those who did not undergo resection due to extensive metastatic burden, rapid disease progression, and reduced performance. The most important determinant of outcome may be the biological behavior of the tumor [64]. In one series the only adverse factor for survival was having an aggressive tumor grade [66]. In an attempt to develop a

metastasectomy-specific prognostic model, the Leuven-Udine group identified primary tumor T stage ≥3, primary tumor Fuhrman grade ≥3, the presence of nonpulmonary metastases, a disease-free interval ≤12 months, and multiorgan metastases as independent pretreatment prognostic factors for survival after metastasectomy in a multivariable analysis [142]. In contrast to the general MSKCC and Heng prognostic models, the results have not yet been externally validated. Currently, evidence stems almost exclusively from retrospective studies and no prospective randomized trials on metastasectomy for RCC have been performed to guide decision making. Though the factors related to prognosis seem to be generally applicable to metastasectomy at any site, some sites may demand specific management strategies, especially when a solitary site if disease or oligometastases are present, and will be discussed in detail.

11.2.1 Other Focal Therapeutic Strategies

Historically, surgical resection has been the preferred approach to metastasectomy, but recent data on stereotactic radiosurgery (SRS) or stereotactic body radiotherapy (SBRT) suggest that this treatment modality is a valid noninvasive alternative [21]. Apparently, the ceramide pathway is activated when high doses of radiotherapy are given per fraction, which lends the stereotactic approach a biological rationale. In addition, an indirect abscopal effect caused by immunological processes induced by a combination of targeted therapy and SBRT is observed. Contrary to surgical metastasectomy, SRS, SBRT, or ablative techniques have been for the most part applied to certain metastatic sites [27]. Although therapy of RCC metastases with SRS is gaining ground and is likely to be expanded to multiple anatomical regions, most of the experience has been gained with treatment of brain and bone metastases and will be discussed under the respective sites. While ablative techniques are minimally invasive, they can still cause bleeding and thermal damage. Cranial and extracranial SRS can induce adverse events such as cough, fatigue, skin rash, and local pain. Side effects are generally frequent, but mild (grades I–II in 96 %) [132].

11.3 Site Specific Metastasectomy

11.3.1 Resection of Pulmonary Metastases

The lungs are the most frequently affected metastatic site with a prevalence rate of 74 % in autopsy studies [116]. Metastasis may be hematogenous or through direct lymphatic drainage of RCC into the thoracic duct which subsequently drains into the subclavian vein and pulmonary artery [8]. There is a wealth of retrospective nonrandomized studies on the resection of pulmonary metastases. Most of these series published until the last decade of the last century were small, with no more than 50 patients [24, 33, 37, 63, 64, 136]. Collectively, in recent series with larger patient cohorts, a 5-year survival rate of 37–54 % was observed in patients with complete resection of solitary or oligometastatic pulmonary metastases [2, 6, 17, 35, 58, 64, 83, 86, 106, 108, 149]. Consistently, several prognostic factors were repeatedly identified in multivariate analyses (Table 11.1). Conversely, incomplete resection was associated with a poorer 5-year survival of 0–22 % [2, 48, 58, 64, 106, 108, 149]. The number of pulmonary metastases removed was associated with survival [2, 17, 35, 48, 64, 106]. In several series, median 5-year survival after complete resection of solitary metastases was 45.6–49 months versus 19–27 months after complete resection of multiple metastases [17, 35, 48]. In the largest reported series, a cutoff was determined with a significantly longer median 5-year survival observed for patients with fewer than seven pulmonary metastases compared with patients with more than seven metastases (46.8 % vs 14.5 %) [106]. Moreover, the presence of lymph node metastases has been associated with shorter survival [6, 106, 108, 149]. In case of simultaneous lymph node metastases, despite complete pulmonary metastasectomy, median survival decreased from 102 to 19 months [149]

and the median 5-year survival rate from 42.1 to 24.4 % [106]. A short disease-free interval after nephrectomy or the presence of synchronous metastases was a consistent factor portending a worse outcome [35, 48, 58, 64, 106, 108]. A disease-free interval of > or <48 months was associated with a median 5-year survival rate of 46 % versus 26 % [35] and a 23-month interval with 47 % versus 24.7 %, respectively [106]. The presence of synchronous pulmonary metastases was a particularly bad feature, with a median 5-year survival rate after complete pulmonary metastasectomy of 0 % versus 43 % for patients with metachronous disease [48]. A further factor is the size of pulmonary metastases [6, 92, 108]. Complete resection of pulmonary metastases of 5 mm was associated with a median 5-year survival rate of 70 % versus 35 % for those with metastases of approximately 45 mm [92]. The type of resection was not associated with survival [17, 86] and ablation techniques may be an alternative to surgical resection in select patients [122]. In 2011 a lung-specific prognostic score was published, developed from 200 consecutive patients with pulmonary metastases [84]. Again, complete resection, size >3 cm, positive nodal status of the primary tumor, synchronous metastases, pleural invasion, and mediastinal lymph node metastases were independent prognostic factors on multivariate analysis. Three risk groups were discriminated with median OS of 90, 31, and 14 months for low, intermediate, and high risks, respectively. This score is not yet externally validated.

11.3.2 Resection of Liver Metastases

Liver metastases occur in 8–30 % of patients with RCC [89]. In an autopsy study hepatic metastases from RCC were observed in 41 % [116], though only in 5 % as solitary metachronous lesion [131]. The main reason for the paucity of reports on liver metastasectomy either by surgery or ablative techniques is the presence of multiple organ metastases generally making further surgical options futile [36]. Moreover, in contrast to solitary pulmonary metastases, it has been consistently demonstrated that liver metastasis carries a poor prognosis [41, 133, 145]. Currently only small retrospective series exist with 13–68 patients which in part suggests that surgical resection may be beneficial in terms of survival [4, 71, 130, 131, 138]. In earlier series median survival following resection of a solitary liver metastasis was 16–48 months with reported 5-year survival rates between 8 and 38.9 % [4, 71, 131, 138]. As has been shown for other metastatic sites, identified prognostic factors include a disease free interval longer than 6–24 months, performance status and completeness of resection. The largest series retrospectively analyzed the outcome of 88 patients with liver metastasis as the only site [130]. Sixty-eight patients underwent resection and were compared to 20 who refused. The median 5-year overall survival rate after resection was 62.2 % versus 29.3 % in the control. In both cohorts 79 % received systemic therapy. This study may indicate that surgical resection of hepatic metastasis is a valuable treatment strategy for carefully selected patients. Patients with high-grade RCC and those with synchronous metastases did not benefit from this approach. Moreover, hepatic metastasectomy was associated with significant morbidity of 20.1 % [130] and one series reported a mortality rate of 31 % [131]. In addition, recurrence frequently occurs after liver resection [4]. These caveats have to be balanced against a potential benefit when selecting patients. It is unclear whether surgery is superior to ablative techniques in this setting [39].

11.3.3 Surgery for Bone Metastases

Skeletal metastases are observed in 16–26 % of patients with metastatic RCC and are frequently symptomatic [89]. The true prevalence of solitary bone metastases is not known. In a series of 94 patients with a solitary RCC metastasis, single bone lesions were observed in 5 patients (5.3 %) [64], and others were observed at a rate of 2.5 % [140]. Although prolonged disease-free survival has been reported after surgical resection of single and even multiple lesions, for most patients

the goal of treatment will be palliative because of pain, nerve root compression, and pathological fractures. In many of these instances, radiotherapy may be equally effective but no randomized data exist specifically for RCC. Outcome of patients treated with surgical resection of skeletal solitary or oligometastatic disease has only been reported in retrospective series. Early reports demonstrated that patients with solitary bone lesions had a better survival when resected [134]. In a series of 38 cases with bone metastases from RCC, 13 evaluable patients had solitary lesions, and these patients had a survival that was longer than the 5-year survival rate of 55 % for the entire cohort [3]. Five-year overall survival rate of five and nine patients with resected solitary bone lesions in other series was 40 % [64] and 54 %, respectively [26]. Conversely, a series including 25 patients with wide resection of a solitary bone metastasis reported a 5-year survival rate of only 13 % [10]. A recent series reported on 125 patients after resection of multiple metastases including 11 with bone as single site (8.8 %) and 4 (3.2 %) with the bone and lung involved [2]. The majority (75.2 %) had more than three metastases removed. For those patients with sites outside the lungs, the 5-year survival rate was 32.5 % compared with 12.4 % among a matched cohort without complete resection. One of the largest series on surgical resection of bone lesions from RCC included a literature review. Taken together the data revealed 5-year survival rates between 35.8 and 55 % comparable to that observed after resection of lung lesions [3]. In addition, patients with peripheral skeletal location of their metastases had a 75 % 5-year survival rate. Collectively, metachronous disease with a long disease-free interval, appendicular skeletal location with wide excision, and solitary metastases were correlated with longer survival [3]. Up to 15-year survival has been described after wide excision of bone lesions [62]. Others added the presence of a clear cell histological subtype and reported that the additional presence of pulmonary metastases did not predict early death with some patients surviving for years after both completely resected pulmonary and bone disease [2, 45]. Similar predictive factors and survival rates were reported in a number of smaller retrospective series [10, 26, 57, 67]. Due to the retrospective nature of these studies and their size and selection bias, the curative effect of resection of RCC bone lesions remains controversial. Conversely, the surgical resection of bone lesions to effectively palliate pain and symptoms from spinal cord compression is undisputed. Randomized studies do not exist for RCC, but a randomized prospective trial in patients with bone metastasis from various malignancies demonstrated that direct decompressive surgery plus postoperative radiotherapy was superior to treatment with radiotherapy alone for patients with spinal cord compression caused by metastatic cancer [103]. Only a minority had RCC bone lesions. In addition, a prospective non-randomized observation study demonstrated that spinal surgery was effective in improving quality of life in patients with extradural spinal bone metastases from various cancers by providing better pain control, enabling patients to regain or maintain mobility, and offering improved sphincter control [50]. Surgery proved feasible with acceptably low mortality and morbidity rates.

From a surgical perspective RCC bone metastases are highly destructive vascular lesions. They pose surgical challenges due to the risk of life-threatening hemorrhage. The largest series reporting on surgical approach and outcome included a total of 368 bone metastases of RCC to the extremities and pelvis [45]. The majority of surgical procedures involved curettage with cementing and/or internal fixation or en bloc resection with closed nailing or amputation in a few. The overall survival rates at one and 5 years were 47 and 11 %, respectively. Fifteen patients (5 %) died within 4 weeks after surgery due to acute pulmonary or multiorgan failure in the majority of cases.

After resection of painful RCC bone metastases, pain was significantly relieved in 91 % of patients, while 89 % achieved a good to excellent functional outcome, and 94 % with metastatic lesions of the pelvic girdle and lower extremities were ambulatory [67]. In addition, wider resection lessened the risk of recurrence at the same location and the need for reintervention [74]. This was a general observa-

tion made in bone metastasis from a variety of cancers where wide excision resulted in better survival and functional outcome than laminectomy [50]. Therefore, surgery for bone lesions should aim at lasting control at the treated site with a durable fixation or reconstruction to prevent reintervention. As the only randomized trial included radiotherapy in both arms, postoperative radiotherapy should be considered [103]. The literature analyzed in the systematic review suggests prolonged disease-free survival after SBRT or metastasectomy of single and even multiple bone metastases [19]. However, no recommendations can be made as to the best treatment modality. A non-comparative study of 48 RCC patients with 55 spinal lesions suggested the effectiveness of SRS [95]. In this study, the 1-year absence of progression rate in the spine was 82.1 %. A 23 % pain-free rate increased to 44 % 1 month and to 52 % 12 months after SRS. Ablative approaches may be an alternative to surgery in selected cases with bulky bone lesions extending to extraosseous regions [47, 143].

11.3.4 Metastasectomy of Brain Metastases

Metastases to the brain occur between 2 and 17 % of patients with RCC and are symptomatic in more than 80 % of cases [75, 77, 117]. If left untreated, median survival was reported to be 3.2 months [22]. After the introduction of noninvasive radiosurgical techniques, craniotomy has lost its preference except for lesions greater than 2–3 cm, rapid onset of symptoms, and cases of large lesions with midline shift [90, 91, 123]. Generally, factors paramount for selecting patients for therapy of brain metastases regardless of the primary tumor site include performance status, extracranial tumor load, and the course of disease summarized in the Radiation Therapy Oncology Group (RTOG) recursive partition analysis (RPA) [38]. Between 70 and 80 % of RCC patients with brain metastases belong to RPA class II (Karnofsky score (KS) >70 %, further extracranial metastases) who have a reported median survival of 4.2 months [16, 91]. In another study including 4,295 patients, the significant prognostic factors for RCC brain metastasis were KS performance status and the number of brain metastases [128]. Those with a KS of 90–100 % and a single brain lesion had a median survival of 14.8 months versus 3.3 months for those with a KS <70 % and >3 metastases. This was observed and confirmed in 138 RCC patients with brain metastases [123]. In a retrospective series of whole brain radiation therapy (WBRT) survival of RCC patients with a single brain metastasis proved to be only 4.4 months, which suggested that aggressive surgical treatment may be superior [152]. A prospective randomized trial of surgery and WBRT versus WBRT alone in 63 patients with brain metastases from various primaries confirmed the superiority of the combination [96, 146]. For patients with extracranial progressive disease, WBRT seemed sufficient. Currently, WBRT is regarded adequate for patients with a poor performance and multiple lesions in whom palliative control of symptoms is warranted. Craniotomy with resection of brain metastases in 50 patients with RCC proved superior to WBRT with a median overall survival of 12.6 months [151]. The addition of postoperative WBRT did not result in a survival difference. However, stereotactic radiosurgery (SRS) can provide effective local control comparable to surgery even for multiple lesions and recurrent metastases [82]. In one series, 85 RCC patients with 376 brain metastases underwent SRS [91]. The median tumor volume was 1.2 cm (range: 0.1–14.2 cm) although 65 % had multiple brain lesions. Median overall survival was 11.1 months after radiosurgery with a local tumor control rate of 94 %. Most patients (78 %) died because of systemic progression. RTOG RPA classes I, II, and III survived for 24.2 months, 9.2 months, and 7.5 months, respectively. In another 69 patient series, the median survival after SRS was 13 months in patients without and 5 months in those with active extracranial disease [120]. It has been argued that survival rates after SRS are inferior to craniotomy, but the size of the retrospective series involving RCC patients with brain metastases and the fact that more patients with a long metachronous interval and fewer brain metastases were candidates for craniotomy [9, 151] do not allow a direct comparison.

11.3.5 Metastasectomy of Adrenal Metastases

Incidence of adrenal involvement has been observed between 3.1 and 5.7 % in nephrectomy series [105, 126, 144] but in up to 23 % of patients with simultaneous metastasis at other sites. Generally, adrenal metastases portend a poor prognosis despite the fact that solitary ipsilateral metastases are often completely resected at the time of nephrectomy. It is unknown whether this is directly correlated to adrenal metastasis or the fact that most patients with adrenal metastases have advanced tumor stages. In 347 patients with advanced stage disease (T3-4N0-1M0-1), adrenal metastases occurred in 8.1 % [144]. Among 56 patients with adrenal metastases, 82 % had pT3 tumors [126]. On multivariate analysis, only the presence of distant metastases, vascular invasion within the primary tumor, and multifocal growth of RCC within the tumor-bearing kidney were identified as independent predictors of the presence of intra-adrenal metastases [69]. While it is beyond the scope of this chapter to discuss the indication for adrenalectomy at the time of nephrectomy for local disease, it is probably true to conclude that the majority of radiographically or clinically apparent ipsilateral lesions are resected at the time of nephrectomy. As a consequence, little is known about the management of isolated, synchronous contralateral and metachronous ipsilateral or contralateral adrenal metastases. Some series on the management of local recurrences included metachronous ipsilateral adrenal metastases [52, 81, 118]. Generally, survival with locally recurrent RCC is poor with a 28 % 5-year survival rate [52]. However, patients who underwent surgical resection had an improved 5-year survival rate of 51 % compared to 18 % treated with adjuvant medical therapy and 13 % with observation alone. Contralateral adrenal involvement, either synchronous or metachronous, seems to be a rare event. In one autopsy series of patients who underwent nephrectomy for RCC, it was observed in 0.7 % [116]. A small series reported the outcome of 11 patients who had surgery for metastatic RCC to the contralateral adrenal gland. Synchronous contralateral adrenal metastasis occurred in two patients. The mean (median, range) time to contralateral adrenal metastasis after primary nephrectomy for the remaining nine patients was 5.2 (6.1, 0.8–9.2) years. All patients were treated with adrenalectomy. Most patients died from RCC at a median of 3.7 (range 0.2–10) years after adrenalectomy for contralateral adrenal metastasis [72]. Two series described another five patients each [65, 99] and collectively some 60 cases are described in the literature [25]. Survival ranged from 8 to 70 months. The factors that affect outcome are uncertain but seem to be correlated to a metachronous interval of >18 months [65]. Based on these data adrenalectomy for isolated metachronous ipsi- and contralateral adrenal metastasis should be recommended because it is associated with long-term survival in individual patients. As for other metastatic sites, ablative percutaneous techniques may be a valid alternative to open or laparoscopic adrenalectomy [148].

11.3.6 Metastasectomy of Lymph Node Metastases

Though not regarded as distant metastatic disease in the TNM classification, lymph node metastases do occur frequently and are associated with a poor outcome that resembles that of systemic disease. In a retrospective series, survival of patients with regional lymph node involvement was identical to that of patients with distant metastatic disease (Pantuck et al.) [100]. In the literature locoregional and distant, mostly mediastinal, lymph node metastases are differentiated and there is evidence that resection of isolated nodes may be beneficial in terms of survival.

Between 58 and 95 % of patients with lymph node involvement have associated hematogenous metastases [34, 107], which is why lymph node metastases are regarded as a significant indicator of systemic disease and adverse prognosis. Patients with pN0 have a 5-year survival of 75 %, versus 20 % for patients with pN+ [100, 101]. However, there is evidence from the literature that patients with a single lymph node metastasis and no metastatic disease can potentially be cured by lymph

node dissection (LND) [100]. The incidence of regional lymph node metastases in patients with renal cell carcinoma ranges from 13 % to over 30 %. However, the true incidence of solitary lymph node metastasis without distant metastatic disease is unknown and seems to be significantly correlated to tumor size. In nephrectomy and autopsy studies, single lymph node metastases were observed in smaller tumors in 3–4.5 % [44, 100, 101]. At autopsy records, a broad variation of the anatomical localization of lymph node metastases was observed [116]. Ipsilateral renal hilar lymph node metastases were found in 7 %, while pulmonary hilar lymph node metastases were found in 66.2 %, retroperitoneal in 36 %, paraaortic in 26.8 %, and supraclavicular in 20.7 % [116]. Single metastases in mediastinal, axillary, supraclavicular, and iliac lymph nodes without any further metastasis were described [49, 56].

In node positive cases, lymph node dissection was associated with improved survival and a trend toward an improved response to immunotherapy [100] (however, patients with regional nodes and distant metastases had significantly inferior survival to those with either condition alone). Lymph node status was a strong predictor of the failure to achieve either an objective immunotherapy response or an improvement in survival when immunotherapy was given after cytoreductive nephrectomy. However, in multivariate analysis, including both clinical and pathologic variables, lymph node status was found to have less of an impact on survival than primary tumor stage, grade, and performance status [100]. The current consensus is that suspicious lymph nodes either at imaging or palpation should be removed during nephrectomy because it was observed that in patients with positive lymph nodes, lymph node dissection (LND) is associated with improved survival when it is performed in carefully selected patients undergoing cytoreductive nephrectomy and postoperative immunotherapy [100]. Even if a survival benefit is doubtful, locoregional LND at the time of nephrectomy may avoid symptomatic local recurrences. There are no data on management of metachronous regional lymph node metastases other than from series reporting on local recurrences [81] but there is a tendency to choose an investigational approach and pretreat these lesions prior to surgical removal (Sect. 11.5.2).

Isolated mediastinal lymph node metastases are more frequently observed in RCC compared to primary tumors from other organs [79, 113, 150]. Lymphatic vessels were found to always connect to the origin of the thoracic duct, some directly without traversing any retroperitoneal lymph nodes [8]. This feature may play an important role in the frequently observed pulmonary and mediastinal metastatic spread in RCC [7].

Cases of patients with resection of isolated mediastinal and intrapulmonary lymph node metastases have been described with disease-free survival of up to 5 years [7, 59]. As these lymph nodes are usually not resected at the time of nephrectomy, these series contain mostly metachronous lymph node metastases. A retrospective analysis of 101 patients who underwent resection of pulmonary metastases specifically evaluated the prognostic value of concurrent hilar and mediastinal lymph node metastases [149]. These data also provide some information on the potential prevalence of lymph node metastases in patients with pulmonary metastatic disease, which was 35 % in this series. Patients with involved lymph nodes had a worse prognosis. Others found lymph node metastases during pulmonary metastasectomy in 20 % and a similar association with poor outcome [6, 106] (see Sect. 11.3.1). With a median survival of less than 2 years, patients with pulmonary metastases and mediastinal lymph nodes may not be candidates for surgical resection, though match paired analysis showed a trend toward improved survival after LND [149]. Despite poorer survival outcome when mediastinal lymph nodes are involved, two recent retrospective series over periods of 11 and 18 years, respectively, support that better long-term survival can be achieved by systematic resection [70, 111].

11.3.7 Metastasectomy of Other Less Frequent Sites

RCC can metastasize to virtually any anatomical location and these have been described in multiple

case reports. Most of these locations are rare, but some are more frequently observed and have resulted in additional information that may guide treatment decisions.

Since 1952, surgery for pancreatic metastases of RCC has been described in 411 patients in 170 publications [137]. A systematic literature search including patients from the authors' institution evaluated the clinical outcome of RCC patients with pancreatic metastases [137]. Evaluable data were retrieved and analyzed for 321 surgically and 73 nonsurgically treated patients. In the resected group, 65.3 % of the metastases were solitary and symptomatic in 57.4 %. After resection the 2-year and 5-year disease-free survival rates were 76 and 57 %, respectively. Two- and 5-year overall survival rates were 80.6 and 72.6 %. After multivariable analysis, the only significant risk factor for disease-free survival was extrapancreatic disease ($p=0.001$). This however had no impact on overall survival in the group of resected patients, which was only adversely affected by symptomatic metastatic disease ($p=0.031$). Interestingly, the interval from primary RCC to pancreatic metastasis and the number of pancreatic lesions were not associated with a worse outcome. Patients with unresected pancreatic disease had significantly shorter 2- and 5-year overall survival rates of 41 and 14 %, respectively. Collectively, these data suggest there is an indication for resection in patients in whom the pancreas is the only metastatic site and who are fit enough to undergo pancreatic surgery. The observed in hospital mortality rate after pancreatic surgery for metastatic RCC was 2.8 % and a significant number of patients underwent extensive surgery with pancreaticoduodenectomy in 108 patients (35.8 %) and total pancreatectomy in 60 (19.9 %). Given the retrospective analysis of various external data and the probability of significant surgical morbidity, it is therefore preferable to start systemic therapy in patients with a short disease-free interval between nephrectomy and pancreatic metastasis. In accordance with the strategy outlined in Sect. 11.5.2, surgery may be reconsidered after a number of pretreatment cycles in those with disease stabilization or shrinkage.

Another uncommon site involves the thyroid gland. Early cases have been described in the 1940s [76]. The largest retrospective series reports on 45 patients undergoing resection of solitary thyroid metastases at 15 different centers, though some patients had resection of other metastatic sites earlier in the course of disease [51]. The 5-year overall survival rate was 51 %. Fourteen patients (31 %) died of disease progression and nine developed a recurrence in the thyroid remnant. In a multivariate analysis, prognosis was significantly worse in patients older than 70 years. The authors described a significant coincidence of thyroid and pancreatic metastases in their series. Of the 45 patients with thyroid disease, 14 (31 %) developed pancreatic metastases. A French group reported on seven patients with solitary RCC metastases in the thyroid, six of whom metachronous after resection of other metastases. The median overall survival after thyroidectomy was 38.1 months [12]. In a clinicopathological study of 36 cases, 23 patients had documented previous evidence of RCC (64 %) as remotely as 21.8 years before the thyroid metastases (mean, 9.4 years). The metastasis to the thyroid gland was the initial manifestation of RCC in 13 patients. Twenty-three patients (64 %) died of disease progression (mean, 4.9 years), but 13 patients (36 %) were alive or had died without evidence of disease (mean, 9.1 years) [42].

Generally, there is little information on how to treat those rare sites. In these circumstances factors associated with a favorable outcome after metastasectomy should be considered for treatment selection (Table 11.1). Individual decisions have to be taken for each case.

11.4 Complete Resection of Multiple Metastases

Complete resection of multiple metastases can be defined as either a resection performed simultaneously at one or more sites or as repeat metastasectomy of asynchronous recurrences after first resection.

The latter reflects a more benign course of the disease. It is therefore not surprising that repeat

Table 11.2 Five-year survival rates after complete resection of solitary or oligometastasis for various sites

Metastatic site	Patient numbers	5-year survival rates	Authors
The lungs	48–149	37.2–54 %	Assouad et al. [6], Kavolius et al. [64], Kanzaki et al. [58], Pfannschmidt et al. [106]
The liver	31–68	38.9–62.2 %	Staehler et al. [130], Thelen et al. [138]
The bone	9–38	13 %*, 40–55 %	Althausen et al. [3], Baloch et al. [10], Durr et al. [26], Kavolius et al. [64]
The brain	11–138	12–18 %	Kavolius et al. [64], Shuch et al. [123]
The adrenal	5–30	51–100 %	Itano et al. [52], Onishi et al. [99]
LN synchronous	129	20 %	Pantuck et al. [101]
LN metachronous	15	63 %	Kavolius et al. [64]
The pancreas	321	57 %	Tanis et al. [137]
The thyroid	45	51 %	Iesalnieks et al. [51]

LN locoregional lymph node metastases

metastasectomy can result in exceptionally long survival lasting more than 10 years in selected individuals [135, 153]. In a series of 141 patients with complete resection of solitary metastases, 5-year survival rates after complete resection of second and third metastases were not different compared with initial metastasectomy (46 and 44 %, respectively, vs 43 % 5-year OS rates; p = nonsignificant) [64]. This is in line with an early retrospective study in which repeat metastasectomy led to longer survival when compared to nonsurgical treatment of recurrence after first metastasectomy [40].

Survival of patients who underwent complete metastasectomy for multiple synchronous RCC metastases at one or more sites has recently been analyzed for a larger series [2]. Of 887 patients with metastatic RCC, 125 patients were identified who underwent complete surgical resection of multiple metastases (2–>3 metastases). Multiple metastases in the lungs as single site were removed in 39.2 %, but 52 % had resection at two or more sites including the lungs, bone, visceral organs, and other locations. Patients with complete metastasectomy restricted to the lungs had a 5-year survival rate of 73 % versus 19 % for those who did not undergo complete resection. Likewise, patients with multiple non-lung-only metastases had a 5-year survival rate of 32.5 % with complete resection versus 12.4 % without. Controlling for ECOG performance status and disease burden those without complete resection had a nearly threefold increased risk of death from RCC. A previous study from the same institution reported on a scoring algorithm to predict cancer-specific survival for patients with clear cell metastatic RCC [73]. Complete resection of multiple metastases was associated with a 50 % decrease in the risk of death on multivariate analysis. Conversely, others reported that patients with metastatic RCC to only one organ site fared significantly better than patients who had evidence of disease in multiple organs (Han et al. [41]). Because of the retrospective, nonrandomized setting of these studies, it cannot be ruled out that multiple metastasectomy benefited patients who would have had a favorable course of disease regardless of surgical intervention. Careful selection of patients with multiple RCC metastases should be made according to general prognostic factors (Tables 11.1 and 11.2).

11.5 Metastasectomy Following Systemic Therapy

11.5.1 Metastasectomy After Biological Response Modifiers

The concept of pretreating patients with metastatic disease followed by complete surgical resection has been investigated in the 1980s and 1990s in small retrospective series. Between 1988 and 1996, 14 patients underwent initial interleukin-2-based cytokine therapy followed by surgical resection of primary and metastatic RCC

lesions [68]. After cytokine therapy, nine patients had an objective response and five patients had stable disease. All patients were then rendered disease-free by surgical excision of residual metastases and the primary tumor. The cancer-specific survival rate at 3 years was 81.5 %. The median overall survival was 44 months (range 4–97 months). Two other series of 16 and 17 patients treated with either interleukin-2 [121] or interferon alpha [119] followed by complete resection of all lesions reported median overall survival of 11 months (range 4–44 months) and 26 months (range 6–34 months), respectively. Another series evaluated this strategy for pulmonary metastasis only and found similar long-term survival [136]. The results of these studies were often used to justify aggressive surgical resection of stable or responding lesions after cytokine therapy, but it has to be acknowledged that these series contained patients with resectable oligometastatic disease that were retrospectively selected because complete resection had been achieved. Only one prospective trial has been performed to investigate if cytokine therapy followed by surgical resection of metastases with curative intent after a period of disease stabilization or response leads to prolonged survival [20]. Within a period of 8 years, 38 patients with responsive or stable potentially resectable metastatic RCC after cytokine treatment were enrolled. Patients subsequently underwent metastasectomy with curative intent and adjuvant systemic therapy. Predictive factors for a favorable long-term outcome included pulmonary disease and surgical complete resection. The median overall survival was 4.7 years (range 3.0–7.8 years) with a median time to progression of 1.8 years (0.8–3.1 years). Twenty-one percent of the patients remained disease-free by the end of the study. Failure to have a surgical complete resection was the strongest negative predictor of prolonged progression-free and overall survival. In addition, metastasectomy of multiple sites if completely resected did not seem to be associated with worse prognosis than of a solitary metastasis. A secondary objective of this small study was to determine the percentage of patients who would achieve complete resection of their metastases considered resectable by radiographic criteria, which was 76 %. Though the trial is limited by its small sample size, it appeared that patients with good performance status, oligometastatic disease regardless of organ site, and a period of disease stabilization or response may be the candidates in whom complete metastasectomy is eventually feasible and associated with long-term survival. This finding supports the results of several previously published retrospective studies.

11.5.2 Metastasectomy Following Targeted Therapy

The higher response rate and downsizing after targeted therapy in comparison to cytokine treatment may increase the therapeutic multimodality options in RCC. As a consequence, more patients who were not candidates for complete metastasectomy or cytoreductive surgery are now being offered systemic therapy with the option to reconsider resection following response or substantial downsizing. To date, this investigational approach has not been prospectively studied, but case reports and retrospective series have been published. This concept may follow distinctively different goals (Table 11.3) (Fig. 11.1).

Several cases have been reported with shrinkage of nodal metastases following tyrosine kinase inhibitors. Sunitinib therapy was followed by complete resection of bulky lymphadenopathy with encasement of the great vessels not amenable to initial excision in a number of patients with a primary clear cell RCC and no evidence of

Table 11.3 Rationale for pretreating patients with targeted agents prior to planned metastasectomy

Turning patients with technically unresectable disease into candidates for metastasectomy after downsizing
Reconsidering patients with multiple and extensive metastases for complete surgical resection after downstaging to oligometastatic disease
Selecting patients who do not progress under therapy for metastasectomy
Improving cancer-related morbidity in patients who may be candidates for metastasectomy but have a reduced performance status

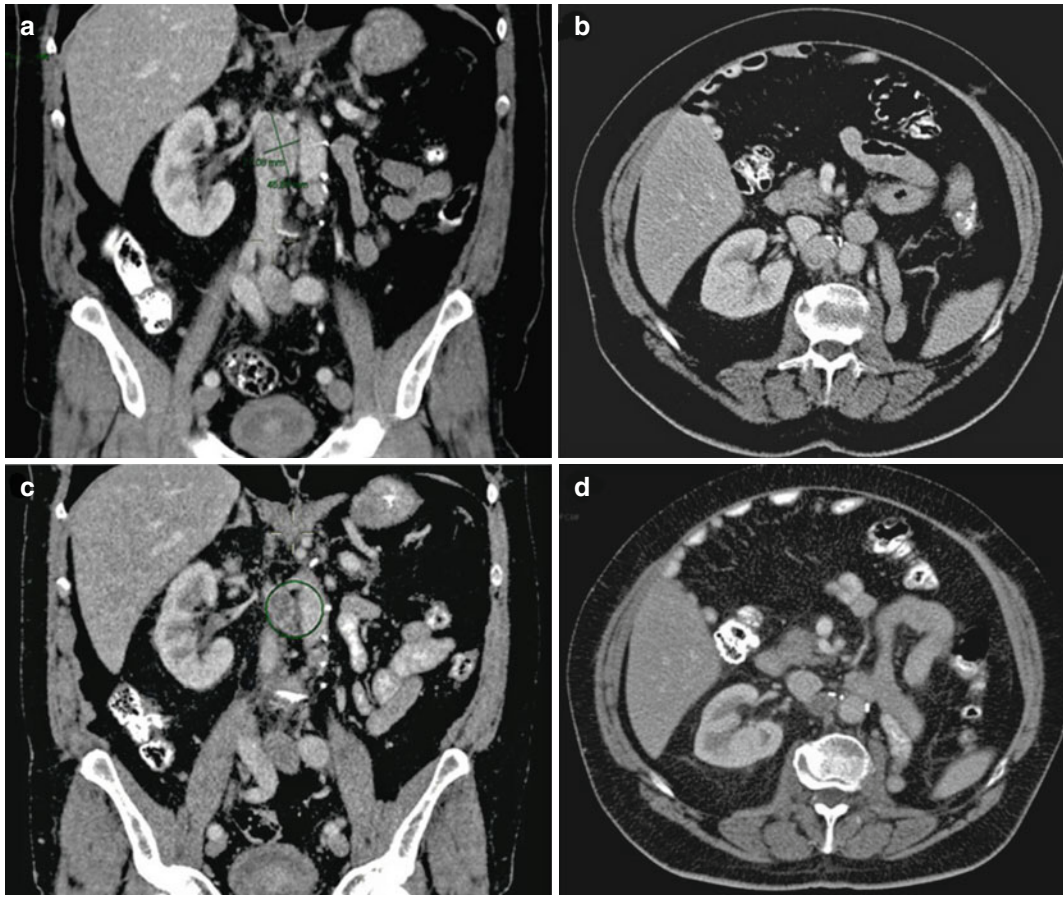

Fig. 11.1 CT scan of a 67-year-old male patient before (**a**, **b**) and after (**c**, **d**) three cycles of sunitinib for metachronous retroperitoneal lymph node metastases 2 years following nephrectomy of a clear cell RCC. The absence of progression under pretreatment and downsizing may be used to select patients for metastasectomy. In this case it remains disputable if retroperitoneal lymph node dissection was facilitated by pretreatment. Despite viable clear cell lymph node metastasis at pathology, the patient remains disease-free at a follow-up of 12 months

distant metastases [102, 112, 124, 139]. In all instances downsizing up to 40 % was reported following five to ten cycles. "Second-look" surgery with complete retroperitoneal LND was feasible in all cases. Despite necrosis all had viable clear cell RCC on pathology. Others have observed prolonged disease-free survival after complete resection of pretreated metastatic lesions at sites other than the retroperitoneum. A series reported on three patients with complete resection of liver, lymph node, and vertebral metastases following the absence of further progression under treatment with sorafenib and sunitinib [129]. The patients remained disease-free after 16, 24, and 29 months. There are reports on the discontinuation of targeted therapy after complete resection of metastatic lesions. A series of patients who discontinued targeted therapy after complete response included six patients after complete resection of residual metastases in the lungs, iliac bone, skin, and thyroid following treatment with sunitinib. The patients remained off treatment for 5–19 months [54, 55]. The largest cohort included 22 patients from three institutions who underwent consolidative metastasectomy after at least one cycle of targeted therapy [61]. Metastasectomy sites included the retroperitoneum in 12 patients, lung in 6, adrenal gland in 2, bowel in 2, and mediastinum, bone, brain, and inferior venal caval

thrombus in 1 each. A total of six postoperative complications were observed in four patients within 12 weeks after surgery, which resolved with appropriate management. Postoperatively nine patients continued with targeted therapy. In 11 patients recurrence developed a median of 42 weeks after metastasectomy. At a median follow-up of more than 2 years, 21 patients were alive and 1 died of renal cell carcinoma 105 weeks after metastasectomy. In these selected patients with a limited tumor burden after treatment with targeted agents, consolidative metastasectomy proved feasible with acceptable morbidity. Though a significant time off targeted therapy and long-term disease-free status can be gained with this approach, it remains unresolved if this is primarily due to the complete resection of metastatic disease, which has been identified as an independent factor associated with prolonged survival, or the combination of surgery and targeted therapy. This approach may not be disputable in those reported cases with technically unresectable disease who were reconsidered for surgery following downsizing. However, there is little evidence how often pretreatment may result in a meaningful downsizing of metastases allowing resection of an initially inaccessible lesion. In a retrospective study, two to six presurgical cycles of sunitinib were evaluated in patients with synchronous metastatic RCC to downsize surgically complex tumors and reconsider resection [14]. The series of ten patients included four patients with bulky retroperitoneal lymph node metastases and encasement of the major blood vessels. In three patients the lesions had an increase of the longest diameter of 13–46 % following sunitinib. Only one patient had a reduction of the longest diameter of 21 %, but despite the downsizing, encasement of vital structures remained and surgery was not reconsidered. Though not directly transferable, more data on downsizing are available for primary tumors. Several authors observed a median reduction of longest diameter in 7–12 % with only 6 % of the patients having a >30 % reduction of the primary tumor diameter [1, 109], though there is evidence that metastatic lesions with their generally smaller volume have a higher overall response rate and shrinkage [109]. Data on combining surgery with targeted therapy are emerging from several retrospective and prospective non-randomized trials and suggest that pretreatment with tyrosine kinase inhibitors which have a generally shorter half life are preferable over anti-VEGF monoclonal antibodies [13]. Reports indicate that pretreatment with sunitinib, axitinib [43, 60], and sorafenib as long as 1 or 2 days before surgery is not associated with a higher complication rate [13, 18, 43, 60, 80, 109]. Currently prospective non-randomized trials evaluate the role of metastasectomy following targeted therapy (NCT00918775).

Clinical Vignette

A 59-year-old man underwent a transabdominal nephrectomy for clear cell renal cell carcinoma pT2b pN0 cM0, grade 2, in April 2002. He then had regular follow-up with CT scans of the chest and abdomen every 6 months in the first 3 years followed by cross-sectional imaging once a year. In August 2006 he was diagnosed with a solitary pulmonary lesion in the upper lobe of the right lung of 5 cm diameter. The patient had a performance of ECOG 0. The management of this patient was discussed in a multidisciplinary tumor board. For patients with metachronous solitary metastases, a survival benefit and even cure have been consistently reported when complete surgical resection was achieved. It is unresolved whether the observed survival benefit is a consequence of surgical intervention or a selection of patients with a more benign tumor biology who because of their prolonged clinical course were considered for surgical resection of their metastases. The best outcome has been observed after resection of solitary or oligo-pulmonary metachronous metastases, especially after a metachronous interval of 2 years and longer, provided complete resection was feasible. The mass was resected and revealed a metastasis of the previously removed clear

cell renal carcinoma. The decision for metastasectomy was taken because the patient had a number of the aforementioned favorable factors associated with prolonged survival after metastasectomy. There is no evidence for adjuvant targeted therapy after complete resection and the patient was followed by regular cross-sectional imaging. He is now disease-free since 8 years and without systemic therapy during the course of his disease.

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Thermal Ablative Techniques in Renal Cell Carcinoma

12

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Contents

Take-Home Points

- Energy ablative therapies are used for treatment of small renal cell carcinomas in patients who are not suitable for surgical resection, who are at risk for multiple renal cell carcinomas, or who refuse surgery.
- Radiofrequency ablation and cryoablation are safe and effective for treatment of small renal cell carcinomas.
- A biopsy should be performed prior to ablation to confirm diagnosis of renal cell carcinoma.
- Follow-up imaging should be performed regularly to evaluate for recurrent or metastatic disease.

12.1 Introduction

Thermal ablation is the destruction of tissue using either heat (radiofrequency, laser, microwave, or high-intensity focused ultrasound) or cold (cryoablation) [1]. Radiofrequency ablation (RFA) and

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_12

cryoablation (CA) are the most commonly used thermal ablation techniques in the management of small renal cell carcinomas (RCC). Ablation can be performed by minimally invasive laparoscopic and percutaneous approaches. There are no prospective randomized data comparing ablation with the gold standard, nephrectomy. In the absence of long-term follow-up data, ablation is reserved for those patients who are unsuitable for surgical resection, are at risk for multiple RCC, or have minimal renal reserve (e.g., a solitary kidney).

This chapter reviews the range of ablation technologies used experimentally and clinically. The clinical approach to RFA and CA of small RCC is outlined. The merits, limitations, and controversies surrounding these two ablation modalities are discussed.

12.2 Energy Ablation Technology

The treatment of RCC is technically feasible using a range of ablation technologies. Clinical experience has been greatest with RFA and CA. The modality of choice often depends on local resources and expertise.

12.2.1 Radiofrequency Ablation

RFA employs high-frequency (300–500 kHz) alternating electrical current transmission into the targeted tissue via needle electrodes to induce ionic agitation and friction resulting in the production of thermal energy that has both a direct cytotoxic effect and indirect ischemic effect on tissue microvasculature. Cell death happens in 4–6 min at tissue temperatures over 50 °C. Immediate cell death occurs at tissue temperatures over 60 °C. Tissue temperatures over 100 °C result in tissue vaporization, gas formation, tissue carbonization, and eschar formation around the electrode, which reduces the efficiency of the treatment. Thus, the goal of RFA is to maintain tissue temperature between 50 and 100 °C, producing coagulative necrosis while minimizing tissue vaporization and charring. Over time, the ablated tissue is replaced by fibrosis [2, 3].

RFA devices may be bipolar or monopolar. In bipolar RFA, a circuit is created with electrodes where the current flows from the generator to the active electrode, through the tumor, to the second electrode, and back to the generator. With monopolar RFA, a circuit is created with electrodes and grounding pads where current flows from an active electrode inserted into the tumor, via the patient's body, to grounding pads on the patient's skin. Monopolar systems are most widely used in the USA. Commercially available RFA systems use either temperature-based or impedance-based ablation algorithms. Temperature-based systems are designed to achieve a target temperature in the tissue surrounding the ablation probe for a determined duration. Impedance-based systems are designed to prevent excessive elevation in tissue impedance around the ablation probe allowing a determined duration of energy deposition while minimizing tissue charring.

The RF electrodes range in size from 14 to 17 gauge. Electrode design can vary from a multi-tined expandable electrode configuration to a simple straight probe in single or triple cluster configuration. Both the RITA StarBurst probe (Rita Medical Systems, Mountain View, CA) and the LeVeen probe (Boston Scientific, Natick, MA) are multi-tined expandable probes that produce teardrop- and discoid-shaped ablation zones, respectively. Probes of different diameters are available and may be deployed in a stepped fashion. The Cool-tip device (Covidien, Mansfield, MA) can be used as a single straight probe or a cluster probe in which three closely spaced straight electrodes are arranged in a triangular configuration. Internally cooled electrodes have chilled saline circulating through an internal lumen, thus minimizing charring at the electrode tip and optimizing energy transmission through the tissues. On the other hand, perfusion electrodes have an opening at the active tip that allows saline to be infused into the tissue during the ablation. This design has also been referred to as "wet RFA." The saline alters the electrical and thermal conductivity of the tissue during ablation thus increasing the ablation zone. Studies have shown "wet" and "dry" RFA systems to be equally effective in achieving cell death [2, 3].

12.2.2 Cryoablation

CA employs alternating cycles of rapid tissue cooling and thawing to produce liquefactive necrosis. Cell death is induced by osmotic effect from extracellular ice crystal formation, direct injury to cell membranes from intracellular ice crystal formation, and ischemic injury to the microvasculature [4, 5]. Compressed gas, usually argon, is injected into the cryoprobe, and as the gas expands, it cools the shaft of the cryoprobe by the Joule-Thomson effect to as low as −190 °C. Thawing is achieved either by turning off the flow of argon and allowing the ice to passively melt or by introducing another compressed gas, usually helium, which when heats up as it expands by the Joule-Thomson effect and thus actively warms the cryoprobe [5]. Depending on the cell type, tumor temperatures between −19.4 and −40 °C are required to bring about cell death [4–7]. On imaging, the edge of the developing ice ball represents a 0 °C isotherm with the −20 °C isotherm several millimeters inside the edge of the ice ball [8, 9].

Cryoprobes come in different diameters (1.4–8 mm), and the ice balls produced vary in shape and size. As treatment efficacy drops off with increasing distance from the probe, a number of probes may be required to cover a tumor zone. Probes should be positioned within 1 cm from the tumor margin and no more than 1–2 cm from each other [10]. The use of multiple probes creates a synergistic effect that results in the formation of an even larger ice ball.

12.2.3 Laser Ablation

Traditionally, laser coagulation was based on Nd:YAG (Medilas Fibertom, Dornier MedTech, Germering, Germany) infrared laser light with a wavelength of 1,064 nm. More recently, diode-based systems (PhoTex 15; Visualase, Houston, Texas) have been introduced into clinical practice [11, 12]. These systems operate in the range of 805–980 nm, use smaller applicators, and create larger ablation zones in shorter periods of time. The energy is delivered via fibers with a flexible diffuser tip. The active length of the tip ranges from 2 to 4 cm. The radiant energy is absorbed by tissue and transformed into heat. Similar to RFA, cell death occurs by a process of coagulation necrosis. When several fibers are used simultaneously, a laser beam splitter can be applied to enable synchronous energy delivery to multiple fibers. Diode-based laser systems are smaller and lighter, and multiple devices can be used to operate several fibers. Newer devices are MRI compatible and consist of a cannulation needle, a sheath, and a laser irrigation catheter. The latter facilitates cooling of the laser tip and prevents direct contact between the laser applicator and the tissues [11]. There is limited experience with this technology for ablation of renal tumors [12–14].

12.2.4 Microwave Ablation

Microwave ablation relies on the emission of electromagnetic waves in the range of 30–30 GHz from applicators placed in tissue. These microwaves agitate water molecules in the vicinity of the applicator, producing friction and heat. As with RFA, once temperatures exceed 60 °C, cell death occurs via coagulative necrosis [15]. There are several systems approved for use in humans in the USA. Equipment consists of a generator and an applicator referred to as an "antenna." The lack of electrical current being transmitted in the patient obviates the need for grounding pads. While there is limited clinical experience with microwave ablative technology, it does offer a number of theoretical advantages over other thermal ablation modalities [16]. Heating is not dependent on conduction from the antenna tip alone but occurs via a direct field effect in all tissues in the microwave field. This allows rapid and uniform heating of the tissues. The evidence for the application of microwave ablation in the kidney shows encouraging early and intermediate results [17–27].

12.2.5 Ultrasound Ablation

High-intensity focused ultrasound (HIFU) delivers targeted ultrasonic energy to tissue at a selected

depth, and this energy is absorbed and converted to heat eventually resulting in coagulative necrosis. If the energy delivered is increased beyond a certain threshold, tissue cavitation, i.e., mechanical disruption, of the tissue occurs [28]. Thermal necrosis depends on ultrasound frequency, exposure time, absorption coefficient, acoustic reflection and refraction, and perfusion rate in the targeted tissue, while cavitation depends on energy pulse length, frequency, and tissue factors. HIFU may be performed laparoscopically or via extracorporeal approach [29, 30]. Some of the major limitations of HIFU when applied to renal tumor ablation are the difficulty of targeting in a mobile organ as well as overcoming the complex acoustic characteristics of intervening tissues when using extracorporeal approaches.

12.3 Selection Criteria

The primary indication for energy ablation of a primary RCC lesion is to eradicate a tumor with curative intent. In addition, ablation for palliation of intractable hematuria has been reported [31, 32].

12.3.1 Patient

Energy ablative therapy should be considered in patients who are poor surgical candidates, those at risk for multiple RCC, patients with limited renal functional reserve, and those who refuse surgical intervention. Poor surgical candidates include those with cardiovascular or respiratory comorbidities that result in an unacceptably high operative risk. The preservation of renal function is paramount in patients with renal insufficiency and those with a solitary anatomic or solitary functioning kidney, and since ablative therapy is nephron sparing, it may help minimize the need for dialysis in the long term [33–38]. A nonsurgical approach is also favored when residual or recurrent disease is identified in the nephrectomy bed.

A genetic predisposition to RCC is present with von Hippel-Lindau syndrome, Birt-Hogg-Dube syndrome, hereditary papillary cell carcinoma, and hereditary clear cell RCC. While many of these patients will ultimately require partial nephrectomy, ablative therapy may prolong the time to resection [39, 40]. In an effort to preserve renal function, synchronous RCCs (sporadic or genetic) may be treated with surgical resection of the larger lesion and energy ablation of the smaller lesion [41].

Given that many patients being considered for ablative therapy have multiple comorbidities, a risk-benefit evaluation should be performed. Patients should have an acceptable functional status. A coagulopathy that cannot be corrected is the only absolute contraindication to ablation therapy.

12.3.2 Tumor

The ideal renal tumor for therapeutic percutaneous ablation is small (≤3 cm), exophytic, and posteriorly located. If tumor eradication is the goal, the disease should be confined to the kidney (T1a). Extension into the adjacent nodes, the renal vein, or inferior vena cava is a relative contraindication to ablative therapy. In patients with an isolated metastasis that is amenable to treatment, energy ablation of the primary may still be considered [42]. Proximity to the central collecting system, bowel, pancreas, adrenal gland, liver, or gallbladder is a relative contraindication to percutaneous thermal ablation of a renal mass, but these structures can often be displaced or protected by various adjunctive techniques [43–46].

12.4 Pre-procedure Planning

Multiple issues need to be taken into consideration when planning an ablation procedure including patient factors, tumor characteristics, ablation modality, approach, and imaging guidance modality.

12.4.1 Patient

All patients should present for pre-procedure clinical assessment prior to intervention. Serum platelets and international normalized ratio (INR) should be determined. Commonly used labora-

tory criteria for ablation include a platelet count greater than 50,000/μL and an INR less than 1.5. Antiplatelet agents are withheld 5 days prior to the procedure. Patients receiving low-molecular-weight heparin have one dose held prior to the procedure. Baseline creatinine and glomerular filtration rate (GFR) should be recorded so that the impact of treatment on renal function can be established.

The patient's ability to lie in the position planned for the procedure, usually prone, should be assessed. If the institutional criteria for conscious sedation are not met, anesthesia assistance should be sought. At our institution, general anesthesia is used because general anesthesia optimizes patient tolerance, allows greater control of respiratory motion when placing the probe, and may facilitate more accurate targeting of the lesion [47]. Renal ablation has been performed as an outpatient setting at many centers, but at our institution, we admit patients for a 23-h observation period [48].

12.4.2 Tumor

One of the ongoing controversies surrounding ablation of RCC is whether a biopsy should be acquired prior to treatment. Though an enhancing renal mass is most often a RCC, the differential diagnosis includes benign entities such as lipid-poor angiomyolipoma, oncocytoma, papillary adenoma, and metanephric adenoma. As the size of a renal mass decreases, the likelihood of a benign diagnosis increases with up to 25 % of renal tumors smaller than 4 cm found to be benign [49]. The Society of Interventional Radiology as well as a consensus panel of urologists recommended performing a biopsy prior to ablative therapy [50, 51]. A clearly negative result eliminates treatment of benign lesions. A positive result provides details of tumor subtype and grade, information that may become relevant should the patient ever require systemic therapy. A positive result is also important for the validation of ablative therapy and in defining the standard of care for small renal masses in the future. Ideally, the biopsy should be performed during a separate encounter so that sufficient time is given for a complete histological evaluation; however, at our institution, for the sake of patient convenience, most patients are biopsied and then ablated in the same session. The biopsy results are then used to personalize the follow-up regimen for each patient.

Once the ablation procedure has been deemed indicated and feasible, the factors affecting technical success should be assessed and optimized. Tumor size and location are the two most important predictors of technical success. Tumors smaller than 3 cm are ideal for ablative therapy [52–54], though larger tumors can also be successfully ablated [55, 56]. Tumor location may be described as exophytic, intraparenchymal, central, or mixed [57]. Exophytic tumors are defined as those with a component extending into the perirenal fat. Parenchymal tumors are defined as those limited to the renal parenchyma. Central tumors are defined as those with extension into the renal sinus fat. Mixed tumors have components extending into both the renal sinus fat and the perirenal fat. Noncentral, particularly exophytic, tumors have the best chance of complete ablation [52].

12.4.3 Cryoablation Versus Radiofrequency Ablation

The relative merits of CA include lower risk of ureteric injury for lesions close to the collecting system, less intra-procedural pain, and more accurate monitoring of treatment efficacy during the procedure [58, 59]. The ice balls created with cryoprobes are easily visualized using cross-sectional imaging. The zone of ablation can fairly predictably be calculated based on the width of the ice ball. While the ablation zone achieved with the RFA electrodes is relatively predictable, noninvasive monitoring of the ablation zone during the procedure is not currently clinically feasible. Hemostasis achieved by cauterizing vessels is the primary advantage of RFA over CA.

12.4.4 Surgical Versus Percutaneous

Both RF and CA have been successfully performed via open, laparoscopic, and percutaneous image-guided approaches. CA was first applied to RCC by

urologists using an open surgical approach following the success achieved in treating prostatic tumors [60]. This has largely been replaced by laparoscopic ablation. A 2008 meta-analysis of 47 studies treating small renal masses using CA or RFA identified laparoscopy as the approach in almost two-thirds of CA cases, while 93 % of RFAs were performed percutaneously [61]. The introduction of lower-profile applicators have led to increased use of percutaneous CA among radiologists.

All patients undergoing open or laparoscopic ablation require general anesthesia. A percutaneous approach is less invasive and may be performed with moderate sedation. It allows faster recovery and is associated with fewer complications [62]. Percutaneous CA has been estimated to be 2.2–2.7 times less expensive than open or laparoscopic procedures [63]. As laparoscopic probes can be used to displace bowel and other structures out of the ablation zone or applicator trajectory, its use is often preferred for ablation of anterior and central lesions. This limitation of percutaneous ablation can often be circumvented with the use of hydrodissection and CO_2 dissection techniques [43–46]. While the use of larger cryoprobes with a surgical approach can facilitate ablation of larger tumors, Lehman et al. reported a significantly higher complication rate of 62 % (13/21) for laparoscopic CA of tumors over 3 cm in size compared with 0 % (0/30) for tumors 3 cm and under ($p=0.0007$) [64]. One of the advantages of a percutaneous approach is the visualization of the whole ablation probe as it is being placed and monitoring of deep structures during the ablation obtained with CT and MRI guidance. With laparoscopic sonography, echogenic shadowing behind the ice ball can limit visualization of the entire ablation zone and adjacent structures [65].

12.4.5 Imaging Modality

Guidance for thermal ablation of RCC may be provided by several imaging modalities. Ultrasound has many advantages. It is relatively low cost, is readily available, and enables real-time imaging. It does not expose the patient to ionizing radiation. The renal mass can be identified in multiple planes by simply angling the probe. Compression can help displace bowel loops out of the applicator trajectory and decrease the skin to target distance. However, ultrasound does have its limitations. Visualization may be limited by patient body habitus, small lesion size, overlying bowel gas, or intervening lung base. The tip of the applicators and the deep aspect of the ablation zone can be difficult to visualize once treatment has begun because of shadowing from microbubbles during RFA or the growing ice ball during CA; thus, some operators will use ultrasound for initial placement of the applicators but then use other modalities, usually CT, for treatment monitoring.

Computed tomography (CT) is the most commonly used imaging modality to guide ablation. It provides excellent visualization of the tumor, the applicators, and the surrounding anatomy. CT fluoroscopy enables real-time visualization of the applicator tip as it is being placed and facilitates precise targeting of the lesion. An initial contrast-enhanced study may be required if the lesion and surrounding normal renal parenchyma are isodense to the renal parenchyma. With CA, the hypodense ice ball is easily visualized. The main disadvantage of CT is the exposure to ionizing radiation for the patient. Radiation exposure also becomes a concern for the operator if CT fluoroscopy is used.

MRI offers superb soft tissue contrast. Multiplanar and real-time imaging can be performed. A combination of T1- and T2-weighted sequences can be used to accurately track the ice ball formed during CA [66]. MRI can also monitor treatment efficacy for RFA by tracking changes in tissue temperature [67]. The lack of ionizing radiation is a significant advantage. Disadvantages include lack of availability, small gantry size, lack of operator experience, the need for MRI-compatible equipment, longer procedure times, and greater cost.

12.5 Techniques

Renal mass ablation can be performed by both laparoscopic and percutaneous approaches. In addition, several adjunctive techniques have been developed to allow ablation of renal masses previously thought to be unamenable to ablation.

12.5.1 Laparoscopic Ablation

Laparoscopic ablation is performed via a retroperitoneal approach for posterior and posterolateral lesions. Anterior or anterolateral lesions are accessed using a transabdominal approach [68]. The ultrasound probe is placed on the side of the kidney opposite to the tumor. A percutaneous biopsy may be acquired using an 18-gauge biopsy needle and coaxial technique under ultrasound guidance. The size and number of applicators used depend on tumor shape and size. The probes are positioned and the treatment is monitored using ultrasound guidance. Most often, the laparoscopic approach is used for CA, and most often, a double freeze-thaw cycle is used [69]. To achieve a 5-mm margin of cell death around the tumor, an ice ball extending 10 mm beyond the tumor margin is desirable [8]. Hemostasis is achieved with direct pressure and hemostatic agents, e.g., Surgicel (Ethicon, San Angelo, TX). The cryoprobe tracks may be embolized with Gelfoam (Pfizer, New York City, NY) or fibrin glue (Tisseel VH, Baxter, Deerfield, IL). The site is observed for bleeding under low insufflation pressures. Gerota's fascia is reapposed. The ports are removed and the port sites closed.

12.5.2 Percutaneous Ablation

Usually, the prone or prone-oblique positions are optimal, though the ideal position can vary depending on the location of the renal mass to be ablated. A pre-procedure study with or without contrast should be performed using CT or MRI. Ultrasound may be used in conjunction with other cross-sectional imaging modalities to target the lesion. Regular monitoring of the ablation zone is performed using intermittent imaging. An ablation margin of 5–10 mm around the tumor is desirable [2]. The zone of ablation covered will depend on the lesion, its proximity to vascular structures, the ablation modality used, and the number, size, and configuration of the applicators. Even with an array of single-tine RF probes, repositioning may be required and overlapping of ablation zones performed. Multiple cryoprobes can be used simultaneously to maximize the zone of ablation. The cryoprobes are placed up to 2 cm apart and up to 1 cm from the tumor margin [70]. An immediate post-ablation contrast-enhanced CT or MRI should be performed to assess the zone of ablation and rule out any complications. This is particularly relevant to RFA for which treatment efficacy is difficult to assess during the procedure [71]. However, care must be exercised in interpreting the immediate post RFA CT. As Javadi et al. showed, contrast medium can leak into the ablation zone immediately after RFA resulting in temporary homogeneous enhancement. The treated area can be better appreciated by identifying the relatively low-density sharply demarcated margins and comparing these with the pre-ablation studies [71].

12.5.3 Adjunctive Techniques

Occasionally, radiologists will perform transarterial embolization prior to percutaneous ablation when hemorrhage poses a significant complication risk [72–74]. In addition, embolization of larger tumors (>4 cm) prior to RFA decreases the perfusion-mediated cooling of the tissues and renders thermal ablation more effective [74].

To reduce the risk of thermal damage to the ureter and renal collecting system during RFA of an adjacent renal mass, retrograde pyeloperfusion with a cooled nonionic solution can be performed [44, 75, 76]. This requires transurethral placement of a 5–6-F ureteral catheter with the tip confirmed in the renal pelvis for infusion and a 14–16-F Foley catheter in the bladder for drainage. The ureteral catheter is removed at the end of the procedure. For CA, Froemming et al. described a probe retraction technique used to protect the ureter [77]. After positioning the cryoprobe, proximity to important structures is assessed using CT. Activation of the probe creates an initial small ice ball that fixes the probe in relation to the tumor and also acts as a point of fixation for manipulation. By manipulating the applicators, the tumor and kidney can be retracted away from the structures to be avoided, e.g., the

ureter. CA can then be resumed with standard freeze-thaw cycles allowing the ice ball to extend distal to the probe tip.

If vital structures lie in the path of the applicator or are contiguous with the proposed ablation zone, noninvasive measures such as changing the patient position or levering the applicator against the skin to lift the tumor off the bowel or vascular structure may be performed. Applicator levering has been reported to increase the tumor to bowel distance by 3–4 mm [78]. The safety margin between the probe tines and the nearest adjacent bowel is 1–2 cm [79].

Hydrodissection or gas insufflation can be used to create a plane between the tumor and other structures [43, 45, 46, 80]. With hydrodissection, sterile liquid is instilled through an 18–21-gauge needle placed between the lesion and the bowel under CT or MRI guidance. For RFA, a relatively nonionic solution, e.g., D5W, should be used. With gas insufflation, gas can be delivered intraperitoneally via needle or laparoscopic port or directly into the perirenal space via needle puncture. Gas has a tendency to dissipate; thus, larger volumes are required compared with liquid. The adequacy of insufflation is best monitored with CT, as gas can obscure the view of the tumor when MRI or ultrasound guidance is used [46].

Interposing angioplasty balloons or esophageal dilator balloons between the tumor or applicator and the structure at risk can also decrease the risk of thermal injury [79]. For angioplasty balloons, an 18–19-gauge needle and 0.035″ wire access should be acquired in the plane in which the balloon is to be placed. The balloon should be placed through a sheath and advanced beyond its desired location. It is easier to retract the balloon into position rather than try to advance the balloon over a wire. Balloon expansion is completed once optimal position is obtained. One of the difficulties with balloon interposition is their tendency to slip away over time. Multiple balloons may be required for adequate tissue separation.

Thermosensors can be placed in cases of endophytic tumors and tumors larger than 3 cm to ensure adequate ablation and to prevent thermal injury to the normal renal parenchyma and adjacent structures. These fiber-optic nonconducting temperature probes should be arranged in a triangulated configuration at the deep and peripheral tumor margins and are advanced into position through a nonconducting sheath. A temperature probe may also be placed in a location where high temperatures are undesirable, e.g., periureteric tissue. Carey et al. reported 100 % primary effectiveness for RFA of 37 tumors 3–5 cm in diameter in which real-time temperature feedback of the ablation zone was used to determine the appropriate treatment endpoint [81]. These independent real-time thermosensors can also be used to determine if and where an electrode needs to be redeployed.

Oblique trajectories should be employed when accessing upper pole masses in an effort to minimize the risk of pneumothorax. Placing the patient in the ipsilateral decubitus position decreases lung excursion on the ipsilateral side and thus reduces the plane of contact between the tumor and the overlying lung. If an infradiaphragmatic approach to the tumor is not possible, another option is to use a technique described by Ahrar et al. whereby a transthoracic approach to upper pole renal masses is created by means of an intentional pneumothorax [82]. This involves placement of a 20- or 18-gauge needle and injecting gas into the pleural space. After completion of the ablation, the pneumothorax is treated with simple aspiration or placement of a small-bore (8–10 French) chest catheter under CT guidance. Alternatively, an iatrogenic pleural effusion may be created by injecting nonionic fluid. This technique allows for precise placement and repositioning of the RF electrode under CT guidance without repeated puncture of the visceral pleura.

12.6 Outcomes

Lack of histological evidence to confirm cell death has been one of the strongest criticisms of ablation therapy, particularly since positive biopsies have been reported in non-enhancing tumors. Currently, treatment success is based almost entirely on imaging findings. Furthermore, outcome data from many studies includes lesions for which no histological confirmation of malignancy

was obtained. A meta-analysis of 47 RFA and CA studies found unknown pathology occurred in 33.5 % of ablated lesions [61]. To circumvent such criticism, we advocate performing biopsy before every renal mass ablation to assure accurate data and also to help in the appropriate management of patients [83].

In the published literature, residual or recurrent disease is usually defined as the presence of nodular or crescentic enhancement in the zone of ablation, especially if it is enlarging [84]. Thus, multiple ablations or reablations may be interpreted as an initial treatment failure. In the 2008 meta-analysis by Kunkle and colleagues, the outcomes of RFA (93.7 % performed percutaneously) were compared with CA (two-thirds performed laparoscopically). Any lesion with evidence of persistent local disease, radiographic or pathologic, was defined as local tumor progression, regardless of the time to reappearance. Repeat ablation was performed more frequently after RFA (8.5 % versus 1.3 %; $p<0.001$), and the rates of local tumor progression were greater for RFA (12.9 % versus 5.2 %; $p<0.001$) [61].

However, these results do not solely address the comparative effectiveness of RFA versus CA but rather also incorporate the results of the technique for ablation, percutaneous versus laparoscopic, as was shown by a meta-analysis of laparoscopic and percutaneous ablations conducted by Hui et al. Outcome measures were defined in terms of primary effectiveness (the percentage of tumors treated successfully by the initial procedure) and secondary effectiveness (the percentage of tumors treated successfully overall, including repeated procedures that followed identification of residual or recurrent tumor). A primary effectiveness of 87 % (95 % CI, 82–91 %) was achieved for percutaneous ablation compared to 94 % (95 % CI, 92–96 %) for a surgical approach ($p<0.05$). The secondary effectiveness was not significantly different between the two groups (percutaneous 92 % versus surgical 95 %). The mean tumor size and the proportion of malignant lesions ablated were significantly greater for the percutaneous group (2.8 cm versus 2.5 cm and 84 % versus 64 %; $p<0.05$) [62].

Thus, the apparent inferior results seen following RFA are due in part to patient selection bias, different approaches, and size, type, and number of applicators. In addition, when comparing the outcomes from percutaneous versus laparoscopic ablation, it should be remembered that these procedures are performed in very different settings. Percutaneous ablations are usually performed in an outpatient suite and most often with moderate sedation. Time constraints, patient tolerance, and respiratory motion may prevent treatment of the entire lesion during a single encounter. Given the minimally invasive nature of this approach and the relatively low risk of complications, some operators may choose to perform ablation in more than one session to treat the entire lesion. Laparoscopic ablations, on the other hand, are more invasive and require general anesthesia and in-hospital stay; thus, the aim is to treat the entire lesion during a single encounter. Performing repeat surgery in the same field is difficult and may have higher rates of complications [85].

Long-term follow-up data is now emerging for both RFA (Table 12.1) and CA (Table 12.2). Ma et al. reported on 52 patients who underwent both laparoscopic and percutaneous RFA with a median follow-up of 60 months. The reported 5-year disease-free survival (DFS) was 94.2 %, overall survival (OS) was 95.7 %, and cancer-specific survival (CSS) was 100 % [86]. Psutka et al. reported the results of 185 patients with biopsy-proven RCC treated with percutaneous RFA with a median follow-up of 6.4 years. The reported DFS, OS, and CSS at 5 years were 87.6 %, 73.3 %, and 99.4 %, respectively [87]. For laparoscopic RFA, Ramirez et al. demonstrated at a median of 4.9 years for 79 patients a 5-year DFS of 93.3 %, an OS of 72 %, and a CSS of 100 % [88]. Best and colleagues described the results of RFA performed for 142 patients with a median follow-up of 54 months. Seventy-two percent of the treated tumors were biopsy-proven RCC. Five-year DFS was 91 % overall and was dependent on tumor size. Tumors smaller than 3 cm had 5-year DFS of 95 %, and tumors 3 cm or larger had 5-year DFS of 79 % ($p=0.001$) [89]. Olweny et al. performed a comparative

Table 12.1 Long-term (≥4 year follow-up) radiofrequency ablation results

Author	Follow-up (years)	Approach	Patients	Tumor size (cm)	Primary effectiveness (%)	Secondary effectiveness (%)	DFS (5-year %)	OS (5-year %)	CSS (5-year %)
Ma et al. [86]	5 (median)	Mix	52	2.2 (mean)	100	N/A	94.2	95.7	100
Psutka et al. [87]	6.43 (median)	Perc	185	3 (median)	87	100	87.6	73.3	99.4
Ramirez et al. [88]	4.9 (median)	Lap	79	2.2 (median)	97.5	100	93.3	72	100
Best et al. [89]	4.5 (median)	Mix	142	2.4 (median)	95	98	91	nc	nc
Olweny et al. [90]	6.5 (median)	Mix	37	2.1 (median)	94.6	97.3	89.2	97.2	97.2
	6.1 (median)	PN	37	2.5 (median)	100	N/A	89.2	100	100
Zagoria et al. [91]	4.7 (median)	Perc	41	2.6 (median)	89.5	100	83	66	97.5
Levinson et al. [92]	5.1 (mean)	Perc	31	2 (median)	87	100	89.2 (6.7 year %)	62.7 (6.7 year %)	100 (6.7 year %)

Perc percutaneous, *Lap* laparoscopic, *PN* partial nephrectomy, *DFS* disease-free survival, *OS* overall survival, *CSS* cancer-specific survival, *nc* noncalculable

Table 12.2 Long-term (≥4 year follow-up) cryoablation results

Author	Follow-up (years)	Approach	Patients	Tumor size (cm)	Primary effectiveness (%)	Secondary effectiveness (%)	DFS (5-year %)	OS (5-year %)	CSS (5-year %)
Johnson et al. [93]	8.2 (mean)	Lap	144	2.3 (mean)	98.6	nc	95.4	90.5	100
Georgiades et al. [94]	5 (mean)	Perc	134	2.8 (median)	98.5	99.3	97	97.8	100
Tanagho et al. [95]	6.3 (mean)	Lap	62	2.5 (mean)	100	N/A	80	76.2	100
Aron et al. [96]	7.8 (median)	Lap	55	2.3 (mean)	100	N/A	86	84	92.5

Perc percutaneous, *Lap* laparoscopic, *DFS* disease-free survival, *OS* overall survival, *CSS* cancer-specific survival, *nc* noncalculable

study of outcomes for two cohorts of 37 patients each undergoing percutaneous RFA versus partial nephrectomy and showed that the 5-year DFS, OS, and CSS were very similar between the two cohorts [90]. Zagoria et al. reported their results for percutaneous RFA performed for 41 patients who were followed for a median of 4.7 years. The 5-year DFS was 85 %, OS was 66 %, and CSS was 97.5 % [91]. Levinson and colleagues related the results of their experience treating 31 patients with percutaneous RFA who were followed for an average of 5.1 years. They reported a 6.7-year DFS, OS, and CSS of 89.2 %, 62.7 %, and 100 %, respectively [92].

CA experience has been greatest using the laparoscopic approach, although long-term percutaneous CA series are also available. Johnson et al. reported on their experience with laparoscopic CA in 144 patients followed for an average of 8.2 years. They reported 5-year DFS of 95.4 %, OS 90.5 %, and CSS 100 % [93]. Georgiades and colleagues reported on their cohort of 134 patients treated with percutaneous CA followed for 5 years. The 5-year DFS was 97 %, OS was 97.8 %, and CSS was 100 % [94]. Tanagho et al. followed 62 patients for a mean of 76 months after laparoscopic CA and found a 6-year DFS of 80 %, OS 76.2 %, and CSS of 100 % [95]. Aron et al. reported 5-year disease-free survival of 81 % in 55 patients with biopsy-proven RCC at a median follow-up of 93 months [96].

Though these long-term data give one greater confidence in the efficacy of thermal ablation for RCC, continued follow-up of these cohorts is necessary because of the known indolent growth rates of small RCCs.

12.7 Post-procedure Follow-Up

Follow-up should encompass an assessment of the patient's clinical status including renal function as well as a review of imaging looking for delayed complications and residual, recurrent, or metastatic disease. A clinic visit should be arranged in the weeks after the procedure to assess for pain, urinary symptoms, fever, or chills. The skin entry sites should be examined.

Given that ablative therapy is advocated in those with limited renal reserve, it is important that the impact of ablation on renal function if any be recorded. Lucas et al. examined the impact of RFA, partial nephrectomy, and radical nephrectomy on renal function in patients with small renal masses (<4 cm). The mean pretreatment GFR was 73.4, 70.9, and 74.8 mL/min/1.73 m^2 in the RFA, partial nephrectomy, and radical nephrectomy groups. Following intervention, the 3-year freedom from stage 3 CKD was 95.2 % for RFA, 70.7 % for partial nephrectomy, and 39.9 % for radical nephrectomy ($p<0.001$). Patients undergoing radical and partial nephrectomy were 34.3 ($p=0.001$) and 10.9 ($p=0.024$) times more likely, respectively, to develop stage 3 CKD compared to RFA counterparts [97]. In patients with a solitary kidney, Raman et al. examined the impact of RFA on renal function in 16 patients with 21 small renal masses (<=4 cm). In this series, the

mean preoperative GFR of 54.2 mL/min/1.73 m^2 declined only to 47.5 mL/min/1.73 m^2 at the last follow-up (mean follow-up of 30.7 months). Patients treated with open partial nephrectomy had a greater decline in GFR compared with those who underwent RFA, at all post-procedure times evaluated: 15.8 % versus 7.1 % at 0–3 months, 24.5 % versus 10.4 % at 12 months, and 28.6 % versus 11.4 % at the last follow-up ($p<0.001$ for all time periods) [34].

There is no standardized follow-up algorithm for ablated renal tumors. The follow-up imaging interval varies among institutions. Matin et al. detected 70 % of incomplete treatments within the first 3 months of treatment. They recommended at least three to four imaging studies in the first year after ablative therapy: months 1, 3, 6 (optional), and 12 [98]. Ideally, follow-up should be performed using the cross-sectional imaging modality used to perform the ablation. Persistent nodular enhancement in the ablation zone up to 3 months post treatment is worrisome for residual disease [99]. Differential diagnosis includes inflammation or volume averaging. Recurrent disease is suspected if the ablation zone is enlarging on serial scans and/or nodular contrast enhancement that was not present on the initial post-ablation study is identified [99]. The renal vein and IVC should be assessed for evidence of enlargement or abnormal enhancement. A search for a new primary tumor and metastatic disease should be performed. Classically, the RFA zone has a "bull's-eye" appearance on surveillance imaging – non-enhancing soft tissue surrounded by enhancing normal renal parenchyma [99]. The ablation zone is usually T2 hypointense compared with the normal renal parenchyma and can have variable intensity on T1-weighted sequences [100, 101]. Subtraction of post-gadolinium and non-contrast T1-weighted data may enhance detection of subtle foci of residual or recurrent disease [102]. While hemorrhage can artificially increase the size of the ablation zone on the immediate post-procedure scan, the lesion should slowly involute to pre-RFA size on serial scans [103] (Figs. 12.1 and 12.2).

During CA, the tumor is frozen and is identified by a well-defined area of low attenuation on CT and is both T1 and T2 hypointense on MRI. While the cryoablated zone is typically non-enhancing on CT and MRI surveillance studies, residual contrast enhancement has been reported [104–106]. In a review of 32 lesions treated with laparoscopic CA, Stein et al. identified persistent ablation site enhancement in 15.6 % (5/32) at 3 months, three of which persisted at 6 months and one displayed enhancement at 9 months. The latter underwent partial nephrectomy that demonstrated no recurrent cancer [104]. The ablation zone is frequently isointense on T1-weighted sequences and hypointense on T2-weighted sequences relative to the renal parenchyma. Involution of the tumor mass on surveillance studies is more prominent following CA due to tissue resorption, than with RFA where the lesion is replaced by scar tissue [99]. Gill et al. reported that tumor size decreased an average of 75 % 3 years post ablation. A further 38 % of cryoablated tumors were not detectable by MR imaging at 3 years (Fig. 12.3) [107].

When recurrence is suspected on follow-up imaging, further management options include active surveillance, repeated ablations, and surgical extirpation. Given that the mean growth rate of small renal masses is 0.13 cm per year, surveillance is reasonable [108]. The majority of recurrences are managed with repeat ablation. Between 7.4 % and 8.5 % of all RF lesions and 0.9 % and 1.3 % of all CA lesions are reablated [61, 109]. In a review of 337 CA patients and 283 RFA patients, Long et al. reported reablation rates of 2.5 % for those who underwent percutaneous CA, 8.8 % for those who underwent percutaneous RFA, and 0 % for those treated with laparoscopic RFA or CA [109]. The inferior results observed with RFA may relate to the inability to precisely monitor treatment efficacy during the procedure compared with CA and perhaps a lower threshold to repeat the percutaneous ablation in the presence of suspicious imaging results. In addition, larger applicators and their placement under direct vision are possible with a laparoscopic approach. Repeat ablations may be performed laparoscopically or percutaneously, although repeat laparoscopic intervention is more challenging. Matin et al. reported 4.2 % incidence of local disease progression after repeat ablations at 2-year follow-

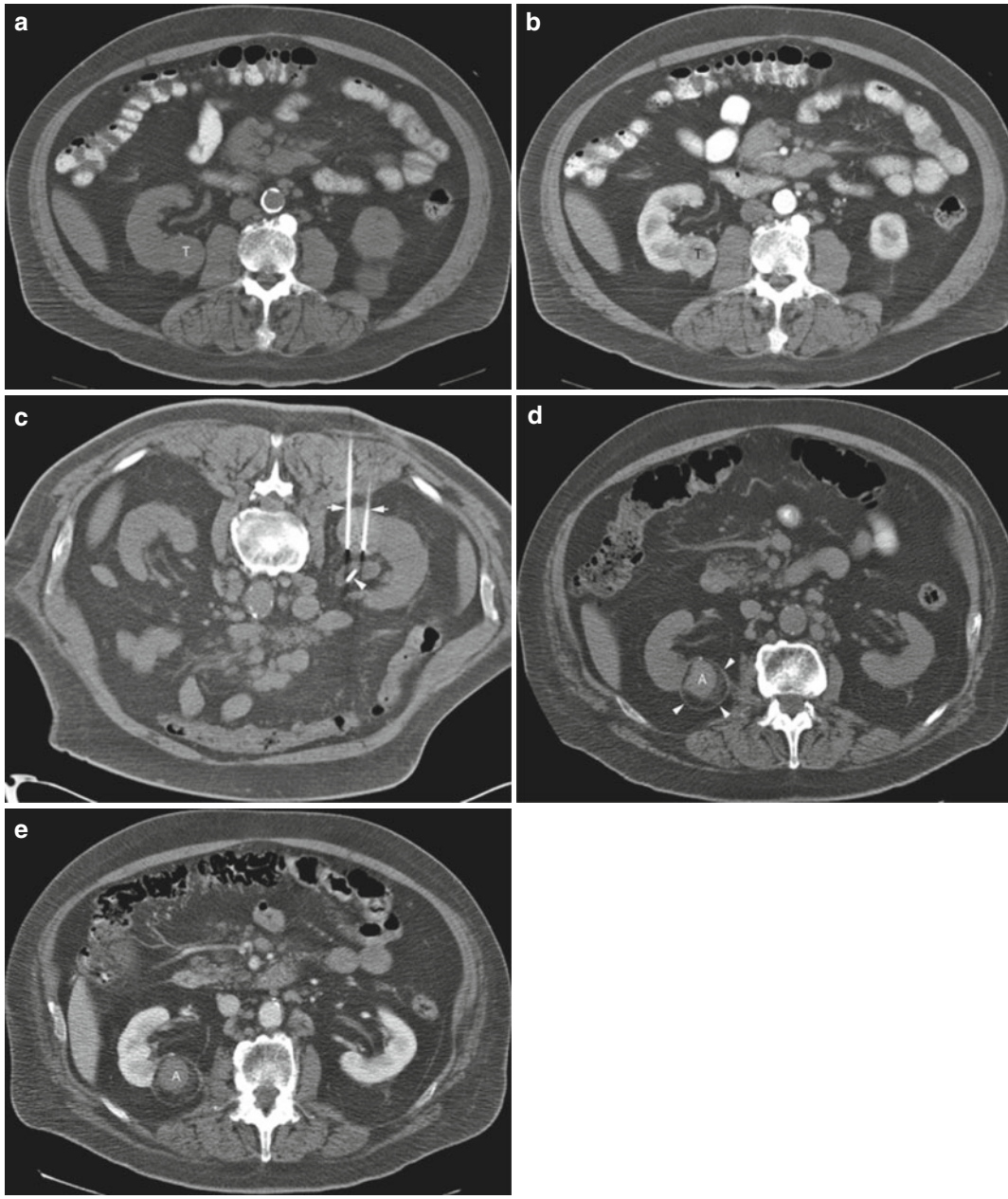

Fig.12.1 A 68-year-old man was found to have a 3.2-cm solid enhancing mass in the right kidney. Biopsy showed renal cell carcinoma, clear cell type. (**a**) Axial CT image of the abdomen without contrast medium shows a tumor (*T*) along the medial border of the right kidney. (**b**) After administration of iodinated contrast medium, the tumor (*T*) shows marked enhancement. (**c**) Axial CT image of the patient in prone position shows two radiofrequency electrodes (*arrows*) entering the tumor from a posterior approach. The tip of each electrode is carefully positioned at the anterior margin of the tumor. A retrograde ureteral catheter (*arrowhead*) was placed for continuous infusion of cold fluid to prevent heating injury to the ureteropelvic junction. Four overlapping ablations were performed to completely ablate the tumor. (**d**) Axial CT image of the abdomen without contrast medium 30 months after ablation shows a soft tissue density at the center of the ablation zone (*A*) surrounded by a fibrous capsule (*arrowheads*). The capsule has engulfed retroperitoneal fat into the ablation zone. (**e**) After administration of the contrast, there is no enhancement of the ablation zone (*A*). A biopsy of the ablation zone (not shown here) demonstrated necrotic tissue and no viable tumor

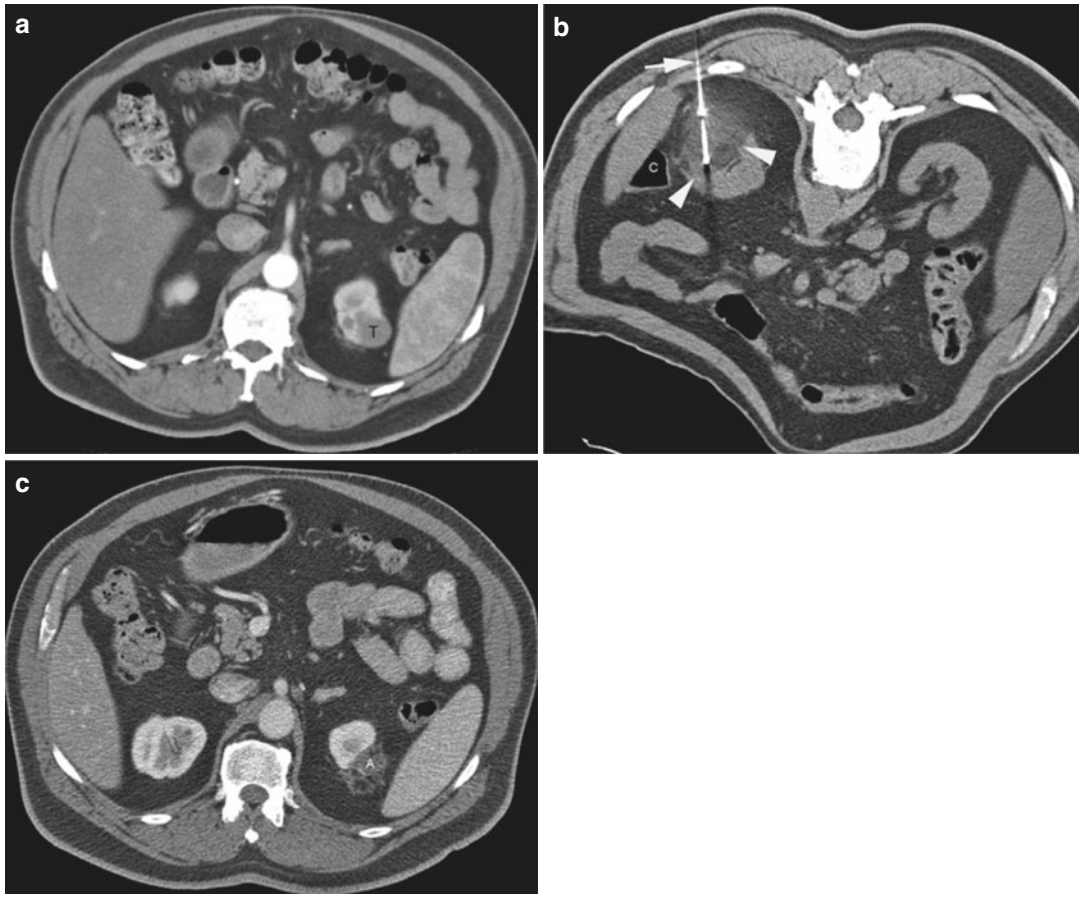

Fig. 12.2 A 62-year-old man underwent CT examination for staging of prostate cancer. He was found to have a 2.7-cm enhancing mass at the upper pole of his left kidney. Biopsy showed renal cell carcinoma, papillary type 1 and Fuhrman nuclear grade 2. (**a**) Axial CT image of the abdomen after administration of contrast shows the tumor (*T*) involving the upper pole of the left kidney. (**b**) Axial CT image of the abdomen in prone position shows one of the three cryoprobes (*arrow*) placed into the tumor from a posterior approach under CT guidance. The ice ball has a lower density compared to the normal kidney. The edge of the ice ball is sharply demarcated at its boundary with normal renal parenchyma. Monitoring the size and extent of the ice ball with CT intermittent CT imaging helps avoid thermal injury to the adjacent structures such as the colon (*C*). (**c**) Axial CT image of the abdomen with iodinated contrast 17 months after ablation shows involution of the ablation zone (*A*) with minimal residual non-enhancing necrotic tissue

up [98]. Salvage nephrectomy is reserved for those in whom reablations have failed or the tumor is too large for reablation. While a surgical resection may be technically feasible, intraoperative and postoperative complications are greater [110].

12.8 Complications

Complications following energy ablation of a renal mass are infrequent and have an incidence of 3–12 % [52, 111–114]. Johnson et al. reviewed complications following 271 RF and CA procedures, both percutaneous and laparoscopic, performed at four institutions. A total of 30 complications (11.1 %) occurred including 5 major (1.8 %), 25 minor (9.2 %), and 1 death (0.4 %). Major and minor complication rates were 1.4 % and 12.2 % for CA and 2.2 % and 6 % for RFA [112]. Atwell and colleagues reported their single institution with 573 percutaneous RFA and CA procedures. They reported 63 overall complications (11 %) including 38 (6.6 %) major complications and no deaths.

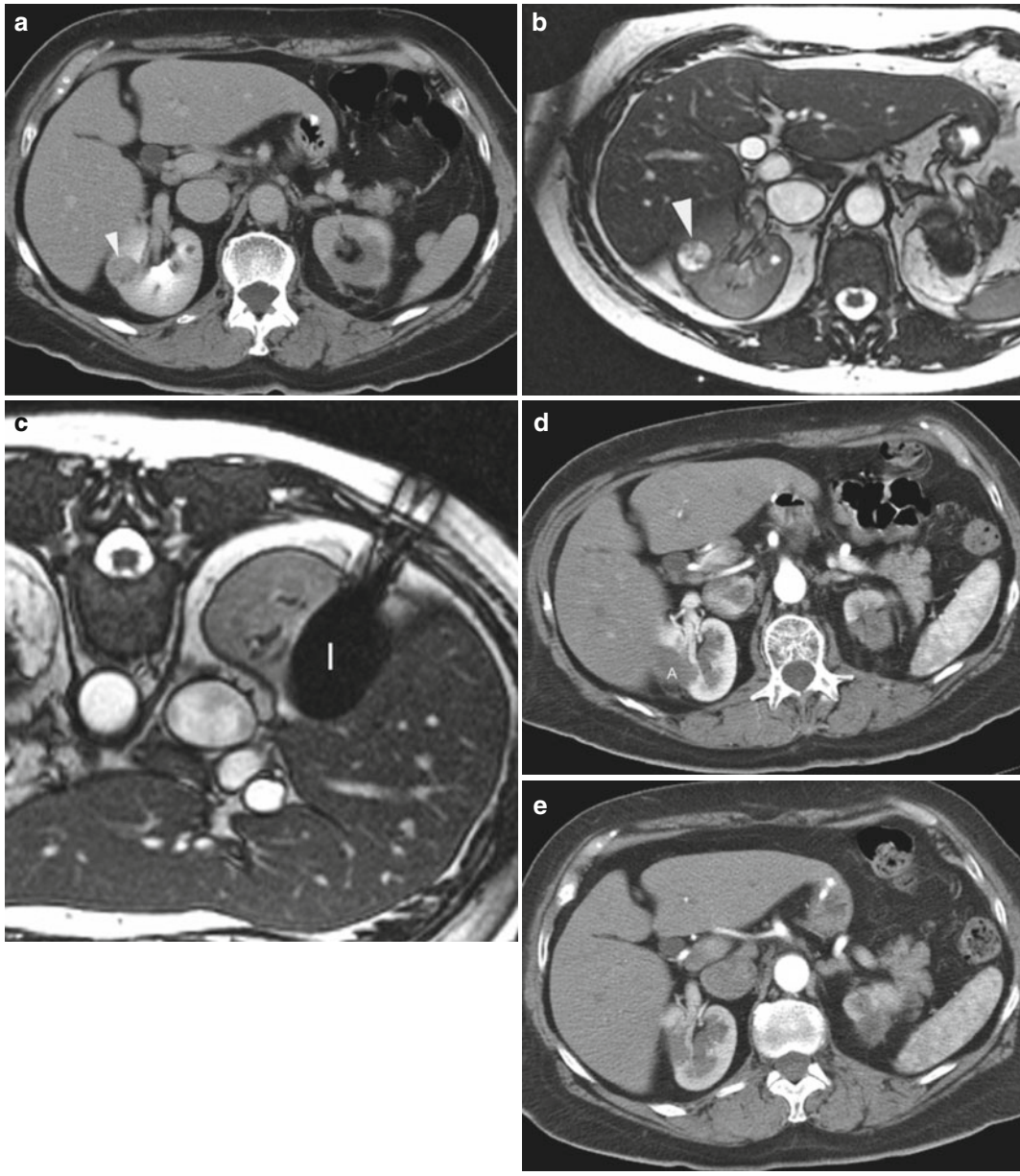

Fig. 12.3 A 65-year-old woman underwent CT imaging for the workup of pancreatic cysts. She was found to have bilateral renal tumors. Biopsy showed renal cell carcinoma, clear cell type and Fuhrman nuclear grade 2 on the right and 1 on the left. Genetic analysis was negative for VHL. The left upper pole renal tumor (not shown here) was treated with percutaneous ablation. (**a**) Axial CT image of the abdomen after administration of IV contrast shows a solid mass (*arrowhead*) in the lateral mid-pole of the right kidney. The tumor was not easily seen on CT images without contrast. (**b**) Axial T2-weighted MRI shows the tumor as a bright, hyperintense lesion (*arrowhead*). She underwent MRI-guided cryoablation of her right renal tumor. (**c**) Axial T2-weighted MR image of the patient in prone position shows the ice ball (*I*) covering the entire tumor. (**d**) Axial contrast-enhanced CT of the abdomen 3 months after ablation shows the ablation zone (*A*) as non-enhancing soft tissue. (**e**) Follow-up CT study at 22 months shows complete resorption of the ablated tumor

Major complication rates were 8.4 % for CA and 4.7 % for RFA, while minor complication rates were 4.8 % for CA and 5.1 % for RFA [114].

Ablation-related injuries are either mechanical or thermal. Structures that are at greatest risk of injury are nerves, vessels, the renal collecting system, and adjacent bowel. Hemorrhage is the most common major complication and is more commonly associated with CA [112, 114]. It usually arises from direct mechanical injury to a vessel by the applicator. The risk is greater with centrally located tumors in which the applicator may traverse numerous segmental vessels en route to the lesion. Bleeding requiring transfusion has been reported in <1 % of RFA and 4.9 % of CA cases. In a review from Lehman et al., major hemorrhage accounted for over 60 % of complications in lesions over 3 cm in size treated via laparoscopic CA [64]. In a retrospective review of 108 percutaneous CAs of lesions over 3 cm, Schmit et al. reported an 8 % major complication rate. Significant hemorrhage following removal of the cryoprobes from the ablated tumor occurred in four of the six patients who sustained a major complication [115]. Cracking of the ice ball with associated parenchymal injury is a recognized, albeit uncommon complication of CA that can result in significant hemorrhage [116, 117]. Potential risk factors include the use of larger-diameter and/or multiple CA probes, initiating a second adjacent ice ball after the primary ice ball had already been formed, and removal of the CA probes before the ice ball has completely thawed [116, 117]. If hemodynamic stability cannot be restored with conservative measures, trans-arterial embolization may be required. Massive hemorrhage due to an arteriovenous fistula is rare but has been described [118]. Bleeding may be avoided by ensuring that coagulopathies and thrombocytopenia are corrected in advance, antiplatelet and anticoagulant agents are held for an appropriate period prior to the procedure, and patient movement is minimized with adequate sedation. Continuous monitoring of the applicator during placement using ultrasound or CT fluoroscopy, ensuring the applicator position is stable before ablation is commenced, can help to minimize hemorrhage. In addition, pre-procedure arterial embolization might also help reduce hemorrhage after CA [73]. Ultrasound or CT imaging of the kidney should be performed at the end of the procedure to rule out bleeding. If ureteral or urethral obstruction with clots occurs, ureteric stenting and/or urinary catheter placement with bladder irrigation may be required.

The incidence of direct thermal injury to the ureter, usually with RFA, has been reported at 1–2 % [52, 111, 114]. Tumors located in the medial aspect of the lower pole are at greatest risk of injury due to their close proximity to the ureter. The risk of ureteral stricture is increased when the distance between the tumor and ureter is less than 2 cm [119]. Retrograde pyeloperfusion using a chilled dextrose solution can help avoid injury during ablation [44, 75, 76]. The trade-off may be suboptimal ablation due to heat sink from the adjacent fluid. CT urography should be performed following ablation if an injury is suspected. The injury can manifest radiologically as ureteral wall thickening, periureteral fat stranding, hydronephrosis, or urinoma. If not promptly identified, acute renal failure can ensue.

Perinephric fat thickness less than 5 mm between the tumor and the bowel is associated with increased risk of thermal injury to the bowel. The risk is greatest with lower pole anterior lesions. Bowel wall thickening is the most likely finding on immediate post-procedure CT. In the weeks after the procedure, the bowel may become adherent to the kidney. Long-term serious sequelae include stricture, obstruction, and perforation. Adjuvant techniques to avoid bowel injury are described in Sect. 12.5.

Pneumothorax has an incidence of 2 % [111]. The risk is greatest with upper pole RCC in which the lung base overlies the proposed electrode trajectory. The majority of cases can be managed conservatively. Moderate to severe pneumothoraces or those associated with new respiratory symptoms may require aspiration and possible chest tube placement. Seeding of the needle track is extremely rare, and enhancing nodules along the needle track often represent inflammatory nodules [120–122].

Conclusion

Partial nephrectomy remains the gold standard for the treatment of RCC. However, RFA and CA have been shown to be safe

and effective treatment options in a select patient population. While the future of these minimally invasive therapies appears promising, the interpretation and validation of the data that exists are fraught with difficulty. Standardization of reporting criteria including clearly defined treatment outcomes and pretreatment histological proof of disease are required to better define the long-term oncologic efficacy of thermal ablation therapies.

Clinical Vignette

A 65-year-old man with history of diabetes mellitus, COPD, and coronary artery disease underwent CT urography without contrast for evaluation of left renal stone. CT study did not show any renal stones. However, an incidental 2.5-cm solid mass was identified in the lower pole of the right kidney. At the time of his presentation, his GFR was 65 mL/min/1.73 m^2. He underwent a contrast-enhanced CT examination for better characterization of the right renal mass. Contrast-enhanced CT confirmed the presence of a 2.5-cm solid mass that showed rapid enhancement after administration of iodinated contrast. There were no suspicious nodes or metastases. Chest radiograph did not show any pulmonary nodules. A preoperative assessment placed him at moderate risk for surgery. He was then referred to interventional radiology for consideration of percutaneous thermal ablation.

A percutaneous CT-guided core biopsy showed renal cell carcinoma, clear cell type and Fuhrman nuclear grade 2. He had normal coagulation parameters including a normal INR and platelet count. He was treated with CT-guided radiofrequency ablation without complications. He was admitted for overnight observation and was discharged home on day 1 following the ablation. He returned to work on post-procedure day number 3.

His follow-up imaging for the first year consisted of CT (renal protocol) at 1, 6, and 12 months. For the second year after his ablation, he had CT scans at 18 and 24 months. These studies demonstrated a non-enhancing zone of ablation that showed some evidence of involution in the first year but remained stable thereafter. A biopsy of the ablation zone at 1 year after ablation showed necrotic tissue and no viable tumor. He will continue to have CT examination of abdomen and chest radiography once a year.

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The Role of Radiation Therapy in Renal Cell Carcinoma

13

Jonathan Verma and Anita Mahajan

Contents

Key Points

- The role for postoperative radiotherapy to the surgical bed in patients with high-risk disease is unclear.
- Radiotherapy is an easy and effective palliative tool for pain control for metastatic RCC.
- Stereotactic radiosurgery is a good alternative to whole-brain radiotherapy or surgery for small medium-sized intracranial metastasis.
- New technical advances allow delivery of high doses of radiation to areas in close proximity to critical structures such as the spinal cord to allow effective therapy for inoperable oligometastatic disease.

13.1 Introduction

RCC has traditionally been considered to be a relatively radio-resistant neoplasm. In a review of *in-vitro* studies, Deschavanne and Fertil found RCC to be the least sensitive to radiation among the 76 included cell types [1]. However, several in vivo studies in mice have suggested beneficial effects from radiotherapy, including a decreased rate of tumor transplantation after radiotherapy [2] and regression of RCC xenografts after

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_13

treatment with radioactive iodine [3]. Clinically, radiotherapy has been shown to be useful in palliation of RCC metastases [4]. Onufrey and Mohiuddin [5] suggested that RCC may respond to higher doses of radiation, measured in time-dose fractionation (TDF) values.

Clinically, radiation therapy only has a minor role in the treatment of RCC. Surgical resection is the major treatment for localized RCC, often with good clinical outcome. None of the available randomized studies have found adjuvant radiotherapy to be associated with a survival benefit, and several found unacceptable rates of serious radiation-related toxicities. For this reason, radiation therapy does not have a role in the management of localized RCC, with the possible exception of unresectable disease in certain cases. Radiation therapy has, however, proven to be useful for palliation of bone and brain metastases in advanced disease.

13.2 Radiotherapy Methods

Radiation therapy can be given in a single treatment (radiosurgery), over a course of three to five treatments (hypofractionated radiotherapy), or over 2–6 weeks (fractionated radiotherapy). The goal of all modern-day radiotherapy technologies is to deliver conformal radiotherapy to the area of concern in a way that will give the lowest risk of morbidity. Numerous different technologies now exist to achieve the goal of conformal radiotherapy using three-dimensional planning approaches. Stereotactic radiosurgery (SRS) refers to the ability to deliver a single radiation dose using an external coordinate system that is referenced to the patient's body. This approach has been employed in the brain with the Leksell Gamma Knife machine (Leksell, Stockholm, Sweden), linear accelerator-based radiosurgery, and the CyberKnife (Accuray, Sunnyvale, CA) technology. Incorporating stereotactic radiotherapy approaches to spine metastasis has increased to the ability to deliver safe and effective high-dose hypofractionated radiation. Intensity-modulated radiotherapy (IMRT) is a computer-based technology that allows very complex-shaped targets to be treated uniformly with excellent conformality of the high dose of radiation to the target while sparing normal tissues that may be near the target area. Any of these technologies can be used to treat a patient for definitive or palliative purposes in the right context.

The most common uses of radiotherapy in patients with RCC are for palliation of symptomatic metastatic lesions and the treatment of brain metastasis. For treatment of symptomatic bone metastasis, the typical course of treatment lasts 2–3 weeks given 5 days a week with each treatment taking 15–20 min. Simple techniques may be used to target many lesions without much discomfort to the patient. For vertebral bone metastasis, stereotactic spine radiosurgery has become a good option for patients with a relatively low burden of disease and a good performance status. This approach delivers one to three fractions of high-dose radiation delivered with a precise technique to minimize the risk of injury to the spinal cord. Results are promising with good symptom and tumor control with minimal side effects [6]. Brain metastasis can be treated with either SRS or whole-brain radiotherapy, with a general preference to use SRS if feasible.

13.3 Localized RCC

13.3.1 Preoperative Radiotherapy

The theoretical benefits of preoperative radiotherapy, as described by Windeyer and Riches [7], include lowering the risk of intraoperative seeding of malignant cells, reducing the size and direct extensions of a tumor, and possibly enhancing the resectability of unresectable tumors. Some of these theoretical advantages are supported by *in vivo* studies in mice [8]. In RCC in particular, pretreatment of xenografts with radiotherapy decreased the rates of transplantation in nude mice [2]. Some authors described small series of patients for whom preoperative radiation therapy seemed to yield improved outcomes [9]. However, the two prospective randomized trials [10, 11] (Table 13.1) undertaken did not find preoperative radiotherapy to be beneficial in all but a very select group of patients.

Table 13.1 Preoperative RT

Author, year	Treatment	Number of patients	5-year survival	Significant difference
Van der Werf-Messing et al. 1981 [12]	RT + N	89	50 %	No
	N	85		
Juusela et al. 1977 [11]	RT + N	38	47 %	No
	N	50	63 %	

The Rotterdam trial [10] compared preoperative radiation therapy followed by nephrectomy to nephrectomy alone. The radiation therapy in this study consisted of a 30 Gy dose in 2 Gy daily fractions delivered to the kidney and regional lymph nodes and was immediately followed by nephrectomy. The study found that preoperative radiation therapy was not associated with any improvement in overall survival or rates of distant metastasis. Local control rates were not reported. The authors did observe that patients with locally advanced (T3) tumors who received preoperative radiotherapy had a lower rate of residual disease after nephrectomy, suggesting that radiation may be successful at making some previously unresectable tumors resectable. However, because resectability was not a primary end point of the study, this conclusion should be taken with some caution. The trial was continued using radiotherapy to 40 Gy, but on subsequent analysis, the higher dose also failed to show any benefit in survival or distant metastasis rate [12].

The Swedish trial [11] was another prospective randomized trial comparing neoadjuvant radiotherapy plus nephrectomy to nephrectomy alone. In this trial, the patients were randomized to receive a preoperative course of 33 Gy delivered in 2.2 Gy fractions followed by nephrectomy or nephrectomy alone. In this study, the patients receiving radiotherapy had a lower 5-year survival, at 47 % vs. 63 % in patients treated with nephrectomy alone, although the difference was not statistically significant.

These trials did have certain limitations. First, the selection of eligible patients may not have been optimal. Both trials included patients of all T stages, including T1 and T2 tumors that are not likely to locally recur after nephrectomy, and neither trials reported local control rates. The only potential benefit to preoperative radiotherapy was a lower rate of residual disease postoperatively, which was not a primary end point of the study. Finally, since RCC is relatively resistant to radiotherapy, doses of 30–40 Gy may not be enough to yield a clinical benefit.

Taken together, these two randomized trials offer evidence that preoperative irradiation does not improve overall survival or diminish rates of distant metastasis in patients with localized RCC and is therefore not indicated in the treatment of the majority of these patients. Preoperative radiotherapy should be considered in patients who have unresectable primary tumors with the goal of making some of these tumors amenable to resection, but this would have to be prospectively validated.

13.3.2 Postoperative Radiotherapy

Early retrospective data [9, 13, 14] suggested that postoperative radiotherapy improved 5- and 10-year overall survival and local control rates. Rafla *et al.* [15] reported improved survival and local control rates at 5 and 10 years, although no details were given on the radiotherapy itself. However, as with preoperative radiation, the two randomized trials [16, 17] (Table 13.2) failed to demonstrate a survival benefit to postoperative radiation. In addition, the studies reported a high rate of radiation-related complications, further discouraging the use of postoperative radiotherapy.

The first study [16] was conducted in Newcastle, UK. Patients were randomized to nephrectomy alone or to nephrectomy followed by radiotherapy, which consisted of 55 Gy in 2.04 Gy daily fractions. The study found no benefit in local recurrence rate in the radiotherapy group and reported inferior overall survival rates in the group receiving radiotherapy.

Table 13.2 Postoperative RT

First author, year		Stages	Tx	Median dose, Gy (range)	Dose per fraction	Number of patients	5-year OS, %	5-year LR,%	Severe toxicity from RT	Conclusions
Randomized										
Finney, 1973 [16]	All	N + RT	55	2.04	52	36	13 % (7/52)	20 (10/52)	No LC or OS benefit, unacceptable toxicity	
		N	–		48	47	15 % (7/48)	–		
Kjaer et al. 1987 [17]	2–3	N + RT	50	2.5	32	38[b]	0 (0/32)	44 (12/27)	No LC or OS benefit, unacceptable toxicity	
		N			33	62[b]	3 (1/33)	–		
Nonrandomized										
Peeling et al. 1969 [47]		NR	N + RT	NR	NR	68	25 (17/63)	NR	NR	No OS benefit
			N	–	–	96	52 (50/96)	NR	–	
Rafla, 1970 [15]		All	N + RT	NR	NR	94	56 (46/81)	7 (7/94)	NR	Significant OS, LC benefit
			N	–	–	96	37 (35/94)	25 (24/96)	–	
Stein[a] et al. 1992 [19]		T2-T4, N0M0	N + RT	46 (36–50)	1.8–2.0	56	50[b]	9 % (5/56)	5 (3/56)	LC benefit in T3 tumors
			N	–	–	63	40[b]	22 % (14/63)	–	
Kao[a] et al. 1994 [21]		3–4	N + RT	45 (41.4–63)	1.8	12	75[b,c]	0 %[b]	0 %	LC benefit, no OS benefit
			N	–	–	12	62[b,c]	30 %[b]	–	
Makarewicz[a] et al. 1998 [20]		T3-T4	N + RT	50	1.8	114	38[b]	14.1[b]	1 (1/114)	LC benefit in T3N0, no OS benefit
			N	–		72	35[b]	20.8[b]		

N nephrectomy, *RT* radiotherapy, *NR* not reported, *OS* overall survival, *LR* local recurrence, *LC* local control

[a]Used CT-based planning

[b]Actuarial rate

[c]DFS

Another randomized trial, conducted by the Copenhagen Renal Cancer Study group [17], compared patients with stage II or III RCC treated with nephrectomy alone or with nephrectomy followed by radiotherapy. In this study, the radiotherapy consisted of 50 Gy in 2.5 Gy fractions, delivered to the surgical bed, ipsilateral, and contralateral lymph nodes. In that study, the adjuvant radiotherapy group had inferior 5-year survival (38 % vs. 63 %). Postoperative radiotherapy did not reduce local recurrence rates; the authors reported very low local recurrence rates in both the nephrectomy and adjuvant radiotherapy groups (0 % and 1 %, respectively). In addition, they reported significant rates of radiation-related toxicity; 44 % of patients were reported to have significant toxicity to the stomach, duodenum, or liver, and radiation-related toxicity accounted for 19 % of the deaths in the study.

These studies had certain limitations. First, the study population may not have been ideally selected to detect potential benefits from radiotherapy. The Newcastle study included a high percentage of patients with T1 or T2 tumors, which have a local recurrence rate of only 5 % after nephrectomy alone [18]. Adjuvant radiotherapy would not be expected to show a benefit in this group but would expose patients to risks of radiation-related toxicity. Several factors may have influenced the high rates of mortality and morbidity of radiation therapy. The Newcastle figures on overall survival included several patients whose deaths were likely not due to radiation (three patients with heart failure and one who committed suicide). The study was also conducted prior to the use of CT-based planning, which aids in minimizing radiation dose to normal structures, thereby lowering toxicity. The Copenhagen group study did use CT planning, but their use of a 2.5 Gy daily fraction size is higher than the norm at most centers and likely contributed to the significant rates of toxicity in their study.

More recently, there have been several retrospective studies [19–21] (Table 13.2) reevaluating postoperative radiotherapy in patients considered more likely to develop local recurrence, including patients with close surgical margins, residual disease, spillage of tumor, or transection of tumor thrombus during nephrectomy [19]. Stein *et al.* reviewed patients of all T stages treated with nephrectomy alone or with nephrectomy followed by elective postoperative radiotherapy consisting of a median dose of 46 Gy in 1.8–2.0 Gy daily fractions. The subgroup of patients with T3 tumors had a reduced risk of local recurrence after postoperative nephrectomy (37 % vs. 11 %, $p<0.05$). However, there was no associated improvement in overall survival, which suggests that local irradiation did not decrease the risk of metastatic disease in this situation. Five percent of patients, all treated without CT planning, had significant small bowel toxicity [19].

Kao *et al.* [21] reviewed 12 patients with T3N0 disease who received postoperative radiotherapy using a median dose of 46 Gy in 1.8 Gy daily fractions. They compared this group to 12 consecutive patients treated with radical nephrectomy alone. Of note, 50 % of the patients receiving postoperative radiotherapy had positive margins versus none in the comparison group. Despite this risk factor, the group receiving radiotherapy had a significantly lower rate of local recurrence (0 % vs. 30 %, $p<0.01$). The disease-free survival rate did not reach statistical significance. Gez *et al.* [22] also found that patients with T3 tumors had a statistically significant lower local recurrence rate (10 % vs. 37 %) after postoperative radiotherapy of 46 Gy in 1.8–2.0 Gy fractions, again with no impact on survival. Noting that the major cause of mortality was systemic relapse rather than local recurrence, the authors concluded that postoperative radiotherapy is not indicated in RCC [22]. Another retrospective study found postoperative radiation therapy reduced local recurrence rates in T3N0 tumors from 15.8 to 8.8 % ($p=0.02$) [20]. Finally, Tunio *et al.* [23] conducted a meta-analysis of seven studies including the ones mentioned above and found that postoperative radiotherapy reduced locoregional failure ($p<0.0001$) but did not affect overall or disease-free survival.

In summary, the literature is somewhat limited on postoperative radiotherapy for RCC. Based on randomized trials finding postoperative radiotherapy confers no survival benefit and is

associated with a significant risk of radiation-related toxicity, radiation therapy is not indicated in the adjuvant setting for localized RCC. However, there is some retrospective evidence that radiation therapy with modern techniques may reduce local recurrence rates in patients with high-risk features for local recurrence, such as positive margins or residual disease, although no survival benefit has been observed in this setting. Further research is needed to evaluate postoperative radiotherapy in patients with high risk of local recurrence.

13.3.3 Stereotactic Body Radiotherapy

There are data that stereotactic body radiotherapy (SBRT) may have a role in the management of RCC. The technique's use of high doses per fraction, typically ranging from 6 to 30 Gy per fraction in contrast to more conventional 1.8–2 Gy fractions, results in a much higher radiobiological dose to the clinical target volume.

Walsh et al. [24] reported that, in a nude mouse model, treating implanted human RCC with 48 Gy delivered over three fractions resulted in tumor shrinkage and marked cytologic changes including decreased mitotic activity and necrosis. There are also some retrospective data suggesting SBRT may be useful in some cases of localized RCC. Beitler [25] reported on nine patients who received SBRT, to a dose of 40 Gy delivered over five fractions, for localized RCC after refusing surgical resection. Four of the nine patients were alive at a median follow-up time of 27 months, and local control was achieved in eight of the nine patients. Wersall et al. [26] retrospectively analyzed 58 patients with RCC, including eight patients who received SBRT for inoperable primary tumors or local recurrences after nephrectomy. These patients were treated with 40 Gy in five fractions, with good results: local control was achieved in seven of the eight patients, with a median survival of over 58 months. Early results of the use of stereotactic ablative radiotherapy (SABR) have shown promising results with reasonable tumor control and kidney sparing for patients who are not surgical candidates or have compromised renal function due to underlying renal disease [27, 28]. In both studies, renal function was maintained in five of the six patients, and all six treated tumors were controlled. Further research is required into the safety and efficacy of SABR/SBRT to evaluate whether it might be a viable treatment option for some patients with RCC.

13.4 Local Therapy for Distant Metastases

13.4.1 Brain Metastases

Brain metastasis occurs in roughly 8–11 % (Saitoh, Rohde) of patients with RCC. Treatment options for RCC brain metastases include surgical resection, radiation therapy with either whole-brain radiotherapy (WBRT) or stereotactic radiosurgery (SRS), or symptomatic management with corticosteroids depending on the clinical situation. The median survival after treatment is typically between 4 and 5 months [29, 30].

WBRT has been shown to successfully palliate neurological symptoms and prolong survival in patients with brain metastases from a variety of solid tumor histologies [31, 32], but it has been somewhat disappointing in the case of RCC. Halperin and Harisiadis found that fractionated radiotherapy of 30–40 Gy was generally unsuccessful at controlling neurologic symptoms from brain metastases or spinal cord compression [4]. Wronski et al. also found unsatisfactory results with WBRT; in their review of 119 patients with brain metastases from RCC, the authors reported median survival of only 3 months after WBRT, with neurologic causes of death in most cases [33].

SRS can successfully control and palliate symptomatic brain metastases from RCC. Sheehan *et al*. reviewed 69 patients who received stereotactic radiosurgery for a total of 146 RCC brain metastases and reported local control in 96 % of patients with follow-up imaging. The authors used a median dose to the tumor margin and its center of 16 Gy and 32 Gy, respectively, and reported that higher doses were statistically related to improved survival [34]. The treatment was also well tolerated, with adverse effects including peritumoral edema in 4.3 %, although one patient did develop

fatal intratumoral hemorrhage. Other studies have reported similar local control rates [35–37].

13.4.2 Bone Metastases

Radiation therapy is useful for pain relief from bony metastases from RCC. Conventional external beam radiotherapy (EBRT) for palliation of bone pain usually consists of 10–20 daily fractions for a total dose of 30–40 Gy [38, 39]. Halperin and Harisiadis reported pain control in 77 % of symptomatic bone metastases [4]. Lee *et al.* prospectively evaluated the efficacy of radiotherapy for pain relief from RCC bone metastases, with the end points of the study being dose of analgesics, patient quality of life, symptoms, and functioning. After treatment with 30 Gy in ten fractions, 83 % of patients in the study reported a decrease in site-specific pain, and 48 % met the study criteria for significant pain response (decrease in pain with no analgesic increase or constant pain with decreased analgesic use) [40]. Similarly, good results with respect to palliation of bone pain have been reported by others [41].

The dose fractionation schedules effective for palliation of bone pain may not be as effective in locally controlling the lesion. Halperin and Harisiadis reported that tumor mass response was observed in 64 % of lesions. Radiation was generally unable to control neurologic symptoms from spinal cord compression, in large part because the limited tolerance of CNS tissue to radiation prohibited administration of a high dose [4].

SBRT, which allows precise delivery of high per-fraction doses of radiation, is another option to treat metastases to the spine, where the proximity of the spinal cord limits radiation by more conventional EBRT. SBRT may also be an option in cases that have not responded to previous EBRT. Yamada *et al.* [42] treated 103 spinal metastases from a variety of solid tumor histologies, including 21 cases of RCC, with a single dose of 18–24 Gy, with excellent results: 90 % of lesions were locally controlled at a median follow-up of 15 months after treatment. Gerszten et al. [43] reported pain control in 89 % of spinal metastases from RCC after 14–20 Gy in a single fraction. In addition, 42 of the total of 60 lesions they analyzed had been previously irradiated with conventional EBRT, with doses thought to preclude further EBRT. Of eight spinal metastases that had progressed after EBRT, seven were locally controlled after SBRT at follow-up ranging from 20 to 29 months. Balagamwala *et al.* [44] report on 57 patients with RCC treated for 88 vertebral lesions with single fraction SBRT with median time to radiographic failure and unadjusted pain progression of 26.5 and 26 months, respectively. This approach offers a rapid, safe, and effective approach for selected patients. However, as more experience is gained, further refinements in patient selection and treatment delivery are being made. Sahgal et al. reported from the experience of 252 patients with 410 lesions treated at multiple institutions. They noted that those patients with lytic lesions, loss of vertebral height, or vertebral misalignment may be a higher risk of vertebral compression fractures after SBRT [45].

Conclusions

In conclusion, radiation therapy is indicated in the management of specific subsets of patients with RCC. In the adjuvant setting, the randomized trials revealed no survival benefit associated with preoperative or postoperative radiation therapy and reported unacceptably high rates of severe radiation-related toxicity. There was evidence that preoperative radiotherapy might occasionally render an inoperable RCC primary tumor operable. There are retrospective data suggesting that postoperative radiotherapy might yield improved local control rates in patients with locally advanced tumors, but no survival benefit has been observed; because radiotherapy carries its own risks, it is not recommended in this setting.

With the advent of advanced planning and delivery of high-dose focal radiotherapy for SABR and SBR, further work to evaluate the abscopal and immunologic effects of localized radiation is warranted. Trials to optimize patient selection, treatment sequencing, and combining

targeted and immunomodulatory agents with radiotherapy should be undertaken [46].

Radiotherapy has been shown to provide palliation of bone pain, and there are data suggesting that SRS can achieve good local control of brain metastases from RCC. Additionally, SRS to focal spinal lesions may be an alternative to vertebrectomy in patients with vertebral body lesions, provided there is no ongoing or immediate threat of neurological compromise. As conformal radiation oncology techniques continue to improve, the role of these techniques in the management of focal lesions in patients with RCC may increase.

Clinical Vignette

In January 2004, a 63-year-old male presented to clinic with gross hematuria and was found to have a large left renal mass, a solitary frontal brain metastases, and pulmonary metastases. He underwent craniotomy for his brain lesion, followed a month later by nephrectomy. He then started interferon therapy and demonstrated slow regression of his pulmonary nodules. A follow-up MRI of the brain a year later demonstrated the development of a new solitary brain metastasis. At this point in time, the patient received stereotactic radiosurgery, with control of the new 0.5 cm lesion. Over the ensuring 2 years, the patient received stereotactic radiosurgery for one additional lesion and was continued on interferon therapy. His systemic disease gradually regressed and became radiographically disease-free. Because of gradually worsening comorbidities, the patient decreased his interferon dose and frequency. Finally, after 5 years of being disease-free, a follow-up MRI revealed three additional new CNS lesions. The patient received stereotactic radiosurgery for these new lesions and continues in follow-up.

This case illustrates the use of stereotactic radiosurgery for the management of oligometastatic central nervous system disease in renal cell carcinoma. This patient was fortunate in being one of the rare complete responders to immunotherapy, but there was no clear concordance between systemic and central nervous system response. It should be noted this patient did not receive whole-brain radiation therapy; this is still a controversial topic in the management of these patients but can potentially be avoided in cases where brain metastases are few in number.

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Part IV

Systemic Therapy Considerations

Adjuvant Systemic Therapy for Renal Cell Carcinoma

14

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Contents

Key Points

- The TNM staging system has evolved to more accurately define risk groups for localized RCC.
- Prognostic systems such as the SSIGN and UISS incorporate clinical variables that are useful in identifying patients at high risk after surgery.
- Surgery alone remains the current standard of care for localized RCC.
- An array of systemic therapies have been studied in the adjuvant setting, including hormonotherapy, chemotherapy, cytokines, vaccines, adoptive immunotherapy, and monoclonal antibodies.
- No adjuvant therapy has yet proven to improve survival after nephrectomy.
- Agents targeting the VEGF and mTOR pathways have revolutionized the management of metastatic RCC. Ongoing adjuvant phase III trials of these agents seek to change the standard of care after surgery.

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14.1 Introduction

Approximately 30 % of patients undergoing nephrectomy for localized renal cell carcinoma (RCC) will end up developing metastases [1, 2].

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_14

Additional therapies to reduce the rate of relapse are needed. As of 2015, surgery alone remains the standard of care for localized RCC, with no adjuvant therapy having a proven survival benefit. The recent development of new and effective systemic therapies for the treatment of metastatic disease holds promise of improving the rates of surgical cure.

Adjuvant therapy is the use of systemic therapy after a local radical treatment in attempt to increase the chance of cure. The rationale for the use of adjuvant systemic therapy is to treat micrometastases early in the disease course in order to have the greatest potential effect in reducing or eliminating future cancer burden. While the ideal goal of treatment should be eradication of micrometastatic disease in order to establish cure and improve overall survival, improvement in disease-free survival is an increasingly accepted end point of adjuvant trials [3]. Several factors are critical in the successful use of adjuvant therapy. First, accurate estimation of the risk of recurrence for an individual patient is necessary in order to decide whether adjuvant therapy is warranted. Second, the chosen agent must have enough activity against the cancer cells in order to affect recurrence. Finally, an ideal adjuvant therapy should have low toxicity and ease of administration in order to promote patient compliance.

A number of randomized adjuvant trials in RCC have been conducted over the past 30 years. First-generation adjuvant studies were conducted prior to the era of targeted therapies and included trials of chemotherapy, hormonotherapy, and immunotherapy. While these were the best available systemic agents at the time, such therapies were minimally effective in the metastatic setting, and the results of adjuvant studies were overwhelmingly negative. With the advent of effective molecular pathway-directed therapies for RCC, we have now entered the era of second-generation adjuvant studies. Vascular endothelial growth factor receptor (VEGF-R)- and mammalian target of rapamycin (mTOR)-targeted drugs have revolutionized the management of metastatic disease and are currently being actively studied in the postoperative, preventative setting. Results are not yet available from many of these new generation trials, and speculation abounds as to whether these new interventions will alter the disease course when administered in the adjuvant setting.

In this chapter, risk assessment strategies for patients in the post-nephrectomy setting will be discussed, as well as a review of the results of first-generation adjuvant studies, and an overview of the ongoing second-generation trials.

14.2 Assessment of Risk

14.2.1 Staging

Proper selection of patients who may benefit from adjuvant therapy is dependent upon an accurate and reproducible assessment of risk. Risk assessment is important for identifying patients with significant enough chance of recurrence to warrant additional treatment while sparing patients at lower risk from the potential toxic effects of adjuvant therapy. The most fundamental yet powerful assessment of risk is determination of tumor stage.

Historic staging systems for RCC include those proposed by Flocks and Kadesky [4], Petkovic [5], and Robson [6, 7]. The Robson criteria were in common use until the development of the tumor, node, metastasis (TNM) system by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) (Table 14.1) [8–11]. While the Robson system focused particular attention on differentiating among tumors with spread beyond the kidney, the TNM system has placed more emphasis on discriminating between intrarenal tumors and is therefore particularly appropriate for use in adjuvant therapy decisions in patients having undergone nephrectomy with curative intent. From its inception in 1978, the TNM renal carcinoma staging system has evolved in an attempt to more accurately distinguish T1 and T2 tumors. The most recent 7th Edition of the TNM system has incorporated further changes to fine-tune risk assessment of tumors confined to the kidney [11]. For example, T2 tumors, previously

Table 14.1 TNM staging systems for RCC since 1987 edition

	T-stage					
Year	T1	T2	T3	T4	N	M
1987 [8]	≤2.5 cm	>2.5 cm	T3a: perinephric or adrenal extension T3b: renal vein involvement T3c: vena cava below diaphragm	Beyond Gerota's fascia	N1: 1 regional node ≤2 cm N2: 1 regional node >2–5 cm N3: 1 regional node >5 cm	M1: distant metastases
1997 [9]	≤7 cm	>7 cm	T3a: perinephric or adrenal extension T3b: renal vein or vena cava below diaphragm T3c: vena cava above diaphragm	Beyond Gerota's fascia	N1: 1 regional node N2: >1 regional node	M1: distant metastases
2002 [10]	T1a: ≤4 cm T1b: >4–7 cm	>7 cm	T3a: perinephric or sinus fat or adrenal extension T3b: renal vein or vena cava below diaphragm T3c: vena cava above diaphragm	Beyond Gerota's fascia	N1: 1 regional node N2: >1 regional node	M1: distant metastases
2010 [11]	T1a: ≤4 cm T1b: >4–7 cm	T1a: >7–≤10 cm T1b: >10 cm	T3a: renal vein or perinephric or sinus fat extension T3b: vena cava below diaphragm T3c: vena cava above diaphragm	Beyond Gerota's fascia	N1: regional nodes	M1: distant metastases

defined as >7 cm and limited to the kidney, have been subclassified into T2a (>7 cm but ≤10 cm) and T2b (>10 cm) based on retrospective data suggesting worse survival for larger tumors within this stage [12]. Tumor size has been found to have a significant correlation with outcome when modeled as a continuous variable, suggesting that any arbitrary size cut-point may be associated with a survival difference if the sample size is large enough [13]. A working knowledge of the changing nomenclature of the TNM system is helpful in interpreting historic adjuvant trials in RCC, which have applied various versions of the staging systems over the years.

Observed 5-year survival rates from the National Cancer Data Base (2001–2002) using the current AJCC staging system are 80.9 % for stage I (T1N0M0), 73.7 % for stage II (T2N0M0), 53.3 % for stage III (N1 and/or T3), and 8.2 % for stage IV (T4 or M1) [11]. It is yet unknown what the impact of multiple new systemic treatments available since 2005 will have on these observed survival rates.

14.2.2 Prognostic Systems

Additional clinical variables have been shown to have prognostic value in RCC beyond TNM stage and include histologic subtype, performance status, Fuhrman nuclear grade, and tumor necrosis [14, 15]. Further refinement of risk has been addressed by the development of several multivariate prognostic systems (Table 14.2). These models differ in their clinical and pathologic covariates, clinical end points, and the constructs of the tool (prognostic category vs. nomogram). The two systems most studied have been the Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) score and the University of California Los Angeles Integrated Staging System (UISS) [16, 17]. The SSIGN system is based on data from 1,801 patients with clear cell RCC and incorporates TNM stage, tumor size, nuclear grade, and histological tumor necrosis to predict cancer-specific survival [16]. The UISS includes three variables as predictors of overall survival for RCC (inclusive of clear cell and non-clear cell): TNM

Table 14.2 Comparison of RCC prognostic systems

Variable	Mayo (SSIGN) [16]	UCLA (UISS) [17]	MSKCC nomogram (all histologies) [22]	MSKCC nomogram (clear cell) [23]	Mayo (Leibovich) [2]
TNM	X (1997)	X (1997)	X (1997)	X (2002)	X (2002)
Size	X	–	X	–	X
Grade	X	X	–	X	X
Necrosis	X	–	–	X	X
Performance status	–	X	–	–	–
Symptoms	–	–	X	X	–
Histology	–	–	X	–	–
Microvascular invasion	–	–	–	X	–

stage, Fuhrman's grade, and ECOG PS [17, 18]. Both systems have been externally validated [19–21]. Two postoperative nomograms have been published by researchers at the Memorial Sloan Kettering: a four-variable system based on data from 601 patients predictive of a 5-year recurrence-free survival and a five-variable system specific for clear cell carcinoma from 701 patients and predictive of a 5-year freedom from recurrence [22, 23]. While these nomograms are useful in predicting risk of recurrence for an individual patient, the SSIGN and UISS systems provide stratification into risk groups which are well suited to adjuvant trial design.

An additional scoring system from the Mayo Clinic was developed to predict progression to metastatic disease as opposed to survival end points [2]. The Leibovich Score incorporates the same variables as the SSIGN system and proposes classification of patients into three risk groups based on score.

14.3 First-Generation Adjuvant Studies

14.3.1 Hormonal Agents and Chemotherapy

Hormonal therapy has been investigated as therapy for RCC based upon the finding of estradiol and progesterone receptor expression on RCC cells [24]. Conflicting results regarding the utility of progestational therapy were reported in early, small, retrospective series [24–26]. A randomized trial of 1 year of medroxyprogesterone as adjuvant therapy was subsequently conducted in Italy, enrolling 136 patients with Robson stage I–III disease [27]. No difference in relapse rate was detected between the treated and observation groups. The 5-year disease-free survival rate was 67.1 % in the medroxyprogesterone group and 67.3 % in the observation group. Side effects included loss of libido in men and weight gain. No significant relationship between sex steroid receptor expression and relapse was detected. Further study of hormonal therapy in the adjuvant setting has not been pursued.

RCC has traditionally been characterized as insensitive to traditional cytotoxic chemotherapy agents. The fluoropyrimidines have been one minor exception to this generalization, with low levels of activity reported in the literature [28]. UFT is a combination of tegafur (a 5-fluorouracil prodrug) and uracil developed in Japan that has predominantly been used in colorectal carcinoma and is approved in many countries outside of the USA. A Japanese single-arm study of adjuvant UFT in combination with vinblastine and doxorubicin reported 96 % 5-year survival among the 31 enrolled patients [29]. A subsequent Japanese trial randomized 71 patients with Robson I–II disease to observation or to 2 years of daily UFT after nephrectomy [30]. No difference in 5-year recurrence rate or overall survival was detected. Side effects were relatively mild and predominantly gastrointestinal in nature. The study included a relatively low risk, early-stage population, as reflected by an 80.5 % 5-year nonrecurrence rate in the UFT arm.

Table 14.3 Randomized adjuvant cytokine studies

Experimental arm	Control arm	N (total)	Stage	End point	Year	Ref.
IFN-α-2b	Observation	247	T3a–b N0 or T2–3 N1–3 (1987)	5-year OS 66 vs. 66.5 % (NS)	2001	Pizzocaro [34]
IFN-α-NL	Observation	283	T3–4 or N1–3 (1987)	OS 5.1 vs. 7.4 years (NS)	2003	Messing [35]
High-dose IL-2	Observation	69	T3b–c–T4 or N1–3 or M1 NED (1997)	DFS 19.5 vs. 36 months (NS)	2003	Clark [45]
IL-2, IFN-α-2a, and 5-FU	Observation	203	T3b–c–T4 or N1–3 or M1 NED (1987)	RFS 4.25 vs. 2.75 years (NS)	2005	Atzpodien [43]
IL-2 and IFN-α	Observation	310	T2–3a–c N0–3 (1987)	5-year DFS 73 vs. 73 % (NS)	2007	Passalacqua [40]
IL-2, IFN-α-2a, and 5-FU	Observation	309	T3b–c –T4 or N1–2 (1997)	3-year DFS 61 vs. 50 % (NS)	2014	Aitchison [44]

14.3.2 Cytokines

For many years, immunomodulatory agents including interferon-α (IFN-α) and interleukin-2 (IL-2) were the basis of treatment for metastatic kidney cancer. Modest survival benefit with IFN was suggested in two randomized trials [31, 32], while the efficacy of IL-2 was evidenced by low but reproducible response rates [33]. High-dose IL-2 remains an option for select patients with metastatic disease based on its association with complete and durable responses in a minority (5–7 %) of patients. Given the vantage of cytokines as the only active therapies for RCC in the 1980s–1990s, a number of randomized trials investigated the adjuvant utility of IFN and IL-2 during this period (Table 14.3).

Several trials have evaluated the efficacy of single-agent IFN given postoperatively. An Italian study randomized 264 patients with Robson stage II–III RCC to IFN-α-2b three times per week for 6 months or to observation [34]. There were no differences in 5-year overall or event-free survival, the primary end points of the study. Subset analysis suggested an improvement in relapse rate among the small number of patients with extensive nodal disease (pN2–pN3) but also suggested a harmful effect of IFN among patients with N0 disease. An Eastern Cooperative Oncology Group/Intergroup trial randomized 283 patients with locally advanced or node-positive disease to 12 cycles of lymphoblastoid IFN-α-NL administered daily for 5 days every 3 weeks or to observation [35]. No statistically significant difference in overall survival was observed, but there was a trend toward better survival in the observation arm (median 7.4 vs. 5.1 years, $p=0.09$).

Combination cytokine regimens incorporating IFN and subcutaneous IL-2 were reported to have greater response rates than single-agent therapy in the metastatic setting [36]. While later randomized studies would fail to show a benefit of combination therapy over single-agent cytokines [37–39], early investigations of combination therapy were undertaken in the adjuvant setting. The Italian Oncology Group for Clinical Research reported preliminary results of a randomized trial of subcutaneous IL-2 and IFN-alpha vs. observation in patients with tumors >2.5 cm and more advanced local disease [40]. This low-dose immunotherapy regimen was given intermittently with twelve 4-week cycles administered over 5 years. This regimen was hoped to be less toxic and with the potential for a prolonged immune stimulatory effect. Approximately one-third of patients were low risk by the UISS system. At a median follow-up of 52 months, there was no difference in RFS (HR 0.81; 0.51–1.27 $p=0.36$) or overall survival (HR 1.07; 0.64–1.79 $p=0.79$).

As discussed above, 5-fluorouracil (5-FU) is one of the few chemotherapeutic agents with a reproducible albeit low response rate in RCC [28]. Some of the highest response rates of the cytokine era were reported with regimens combining IFN and IL-2 with 5-FU [41, 42]. The German Renal

Carcinoma Chemoimmunotherapy Group conducted a randomized adjuvant trial using this approach in patients with tumor extending into the renal vein or invasive beyond Gerota's fascia, node-positive patients, and patients after complete surgical resection of solitary metastatic disease [43]. Two hundred three patients were randomized to 8 weeks of treatment with subcutaneous IL-2, IFN-α-2a, and 5-FU or to observation. The primary end point was relapse-free survival. No significant difference was seen between the treatment and observation arms. Overall survival was significantly decreased in the treatment arm compared with the observation arm (5-year survival 58 vs. 76 %; $p=0.0278$). While no mention of side effects was reported in this publication, the possibility that treatment-related toxicity contributed to the worse survival must be considered. A second randomized trial using a very similar regimen was conducted by the EORTC and NCRI (UK) [44]. Three hundred nine patients with locally advanced or node-positive disease or exhibiting positive microscopic margins or microscopic vascular invasion were randomized to either observation or subcutaneous IL-2, IFN-α-2a, and 5-FU. There was no significant difference in the primary outcome measure, disease-free survival at 3 years (50 vs. 61 %), nor in overall survival at 5 years (63 vs. 70 %).

High-dose, human recombinant IL-2 was the first agent approved for metastatic renal cancer in the USA based on non-randomized, pooled data from 255 patients yielding a response rate of 15 % (95 % CI, 11–20 %) including 7 % complete responders [33]. While the complete response rate in the metastatic setting would suggest potential utility as adjuvant therapy, the significant side effect profile of high-dose IL-2 therapy precludes the ability to conduct a blinded study, poses difficulty in subject recruitment, and greatly limits its widespread use as an adjuvant. An attempt was made by the Cytokine Working Group in studying one course of high-dose IL-2 in the adjuvant setting. This was a randomized trial with observation as the control arm [45]. The trial included patients with locally advanced tumors and was expanded to include patients with M1 disease resected to no evidence of disease. The study was closed for futility after interim analysis suggested minimal likelihood that the study would meet its primary end point of a 30 % absolute improvement in disease-free survival. While side effects were as expected, 88 % of patients in the IL-2 arm experience grade 3–4 toxicity including hypotension requiring vasopressor support in 52 %.

Given the remarkable ability of high-dose IL-2 to occasionally induce complete and durable responses, its use as an adjuvant therapy remains a provoking concept. However, further investigation of the drug in the adjuvant setting would necessitate the existence of a reliable method of predicting responders in order to limit the exposure of those unlikely to benefit. Unfortunately, the ability to identify such patients to a high degree of certainty in the metastatic arena remains an enigma [46].

14.3.3 Adoptive Immunotherapy

Adoptive immunotherapy involves the harvest of a patient's T lymphocytes and ex vivo activation, followed by reinfusion in attempt to engender an immune response against the tumor. The use of this technique as adjuvant therapy was studied in a small, randomized study in patients with node-positive disease after nephrectomy. Forty-five patients were randomized to adjuvant therapy with ex vivo activated T cells plus cimetidine (to reduce in vivo suppressor T-cell function) or to cimetidine alone. The median time to recurrence was 16.4 months for the adoptive immunotherapy-treated patients and 6.5 months for controls ($p=0.0360$) [47]. A subsequent 100-patient phase II trial of adjuvant activated T-cell therapy in high-risk patients (including metastatic patients resected disease-free) showed favorable survival compared to institutional historical controls [48]. Despite these promising preliminary results, adoptive immunotherapy has not been pursued further in definitive studies.

14.3.4 Vaccines

Autologous vaccination strategies are based on the premise that RCC cells express antigens capable of eliciting a T-cell response. The sensitivity of

Table 14.4 Randomized adjuvant vaccine strategies

Experimental arm	Control arm	N (total)	Stage	End point	Year	Ref.
Autologous tumor with BCG + progestogen	Progestogen	43	Not specified	3-year PFS 54 vs. 34 % (NS)	1987	Adler [50]
Autologous tumor with BCG	Observation	120	T1–3 or N+ (year not specified)	5-year DFS 63 vs. 72 % (NS)	1996	Galligioni [51]
Reniale (autologous tumor lysate)	Observation	558	T2–3b N0–3 M0 (1993 suppl)	5-year PFS 77 vs. 68 % ($p=0.0204$)	2004	Jocham [52]
Vitespen (autologous tumor HSP–peptide)	Observation	818	T1b–4 or N1–2 (2002)	Recurrence 37.7 vs. 39.8 % (NS)	2008	Wood [57]

metastatic renal cancer to immunostimulatory interventions such as cytokines is evidence of the immunogenic nature of RCC. Vaccine approaches in RCC have included whole-cell vaccines, lysates of cancer cells, and heat-shock proteins [49]. The post-nephrectomy setting – when tumor burden is at its lowest and the immune system has potentially been relieved of suppression – may be the most opportune time to instigate an immune response through vaccination. The appeal of tumor-derived vaccine strategies has led to a number of such trials in the adjuvant setting (Table 14.4).

An early report of adjuvant tumor vaccination strategy investigated autologous irradiated tumor cells admixed with BCG administered by intradermal and endolymphatic injections [50]. This trial included 43 post-nephrectomy patients of all stages who were randomized to either hormonotherapy with a progestogen (Primostat) or to hormonotherapy in combination with the vaccine. While there was a trend toward improved disease-free interval in the vaccinated patients, no statistically significant difference was seen in this small study.

Another such "active specific immunotherapy" approach was reported by Galligioni et al. [51]. Patients with pT1–3b pN0 or pN+ disease at nephrectomy were randomized to immunization ($n=60$) or to observation ($n=60$). The vaccine was prepared by irradiation of autologous tumor cells and was mixed with Bacillus Calmette–Guèrin (BCG) for the first two of three vaccinations. After a median follow-up of 61 months, there was no difference in 5-year disease-free survival or overall survival between the two groups. Delayed-type cutaneous hypersensitivity response to autologous tumor cells was 1 month after immunization was detected in 70 % of patients, but was not observed in control patients.

Favorable results have been reported in trials using an autologous tumor-derived lysate vaccine (Reniale) developed in Germany [52]. This process involves obtaining tumor cells at the time of nephrectomy followed by incubation with IFN-γ and devitalization by rapid repeated freezing. A large series of T2–3N0 patients received adjuvant therapy with the vaccine in initial studies, with higher 5-year progression-free survival and overall survival rates as compared with historical controls [53, 54]. A subsequent randomized trial was performed to confirm the activity of the vaccine in post-nephrectomy patients as compared to observation [52]. Those randomized to the vaccine received an intradermal injection every 4 weeks for a total of six injections. The primary end point of the trial was progression-free survival. Among 379 patients evaluable for the intention-to-treat analysis, the risk of tumor progression was significantly less in the vaccine group (HR = 1.59, $p=0.0204$). The majority of patients were N0 (96 %), and only 30 % had T3 disease. Subgroup analysis revealed the greatest potential benefit among patients with T3 tumors. Several methodological flaws limit interpretation of this study: randomization was performed prior to nephrectomy, and as a result, 32 % of enrolled subjects were lost prior to starting treatment leading to an ultimate imbalance in the study arms. A follow-up intent-to-treat analysis of 477

patients did not indicate an overall survival advantage ($p=0.1185$), although a secondary per-protocol analysis of 352 patients did suggest an overall survival benefit ($p=0.0356$) [55].

Subsequent to the randomized trial, data from a compassionate use program with Reniale were analyzed to estimate potential survival benefit [56]. Six hundred and ninety-two patients with T2–3 N0–2 M0 (1992 classification) disease who had been treated with the vaccine between 1993 and 1996 were matched with 661 controls who had undergone nephrectomy between 1992 and 2006 at a single center in Germany. The matching criteria included a number of prognostic variables including pT stage, but tumor size was not used due to missing data. Seventy-nine percent of patients had pT2, and 21 % had pT3 disease. Ten-year survival was 69 % in the vaccine group compared with 62 % in the control group ($p=0.066$). On subgroup analysis, improved survival was seen among patients with pT3 tumors ($p=0.022$) but not among those with pT2 disease ($p=0.365$). On multivariate analysis of the whole study group, treatment with the vaccine was associated with improved survival (HR = 1.28, $p=0.030$). Interpretation of these data is limited by the retrospective nature of the analysis, selection of controls from a single institution, and the absence of one important prognostic factor (tumor size) from the matching criteria.

Vitespen (Oncophage) is a heat-shock protein (glycoprotein 96)–peptide complex derived from autologous tumor. Heat-shock proteins are involved in protein folding and are upregulated in response to stress. They bind cellular peptides and are highly immunogenic. In a study of 818 patients with cT1b–T4 N0 M0 or N1–2 M0 clear cell RCC, patients were randomized to vitespen or observation [57]. Vitespen was administered by weekly intradermal injections for 4 weeks, followed by every 2-week injections until depletion of vaccine supply or disease progression. Among 728 patients included in the intent-to-treat analysis, no difference was seen in recurrence rate between the vitespen (38 %) and observation (40 %) groups after a median follow-up of 1.9 years (HR = 0.923, 95 % CI 0.729–1.169; $p=0.506$). Subgroup analysis suggested a trend toward improved relapse-free survival in patients with stage I–II disease, with recurrence noted in 15 % of vitespen-treated patients and 27 % of observation patients (HR = 0.576, 95 % CI 0.324–1.023; $p=0.056$). No overall survival difference was seen after an additional 17 months of follow-up with approximately 88 % patients alive in both groups. The trial had a number of limitations, including the inability to prepare a vaccine for 8 % of patients and a large number of subjects who were not eligible upon blinded review of the intent-to-treat population. Exclusion of these subjects in a full analysis data set resulted in a greater difference in outcomes between the vitespen and control groups but still did not meet statistical significance. Longer-term follow-up of 294 of the patients enrolled in a follow-up registry continued to demonstrate a trend toward improved outcome in lower-stage disease but without statistical significance [58].

14.3.5 Monoclonal Antibodies

Carbonic anhydrase IX (CAIX) is a transmembrane enzyme that catalyzes the conversion of carbon dioxide and water to carbonic acid and plays an important role in proton flux and cellular pH regulation. CAIX is under regulation by hypoxia-inducible factor-1α (HIF-1α) and is highly expressed on the surface of clear cell renal carcinoma cells due to downstream effect of pVHL dysregulation [59]. cG250 (girentuximab) is a monoclonal antibody with a high affinity for the CA IX antigen that can induce antibody-dependent cellular cytotoxicity and elicit lysis of RCC cells [60]. Phase I and II trials of weekly cG250 infusions in metastatic RCC patients demonstrated that the antibody was well tolerated with prolonged stable disease and late clinical responses noted in some patients [61, 62]. Based on these observations, a randomized, placebo-controlled trial of 24 weeks of cG250 in the adjuvant setting was conducted [63]. This large trial enrolled 864 patients with T3–4 N0 or T1b–2 N0 high-grade disease as well as N+ patients with a primary end point of disease-free survival. No difference was seen in either disease-free

(HR = 0.97, $p = 0.74$) or overall survival (HR = 0.99, $p = 0.94$). Subgroup analysis suggested that patients with high CAIX antigen expression had improved disease-free survival with cG250 treatment.

14.3.6 Antiangiogenic Therapy

The resurgence of thalidomide as an anticancer agent based on its antiangiogenic and immunomodulatory effects warranted evaluation in RCC. Several studies in the metastatic setting suggested a disease-stabilizing effect [64, 65]. In the adjuvant setting, a small trial from MD Anderson randomized 46 patients with pT2 (Fuhrman grade 3 or 4), pT3a–c, T4, or N1–2 disease to thalidomide or observation [66]. Thalidomide was administered to a target dose of 300 mg/day for 2 years. The trial was closed to further accrual after a preplanned interim analysis revealed inferior 2- and 3-year probabilities of relapse-free survival in the thalidomide arm as compared with controls (47.8 % vs. 69.3 % and 28.7 % vs. 69.3 %, respectively; $p = 0.022$). While 19 % of thalidomide-treated patients experienced grade 3 adverse events, dose reductions were required in most patients, and only 36 % completed all planned therapy with frequent dropouts due to side effects.

While thalidomide has not proven to be a significantly effective therapy in RCC, targeting angiogenesis through modulation of the VEGF and mTOR pathways has subsequently revolutionized treatment of advanced RCC. The use of these antiangiogenic strategies in the adjuvant setting is the subject of the next section.

14.4 Second-Generation Adjuvant Studies

Once considered among the least treatable of advanced malignancies due to a lack of effective systemic treatments, metastatic RCC has evolved in recent years into a disease that can be managed through effective disease stabilization. This has been made possible by an understanding of the dependence of RCC on the VEGF and mTOR pathways, targeting of which can render RCC susceptible to drug therapy. Seven agents were approved in the USA for the treatment of metastatic RCC between 2005 and 2012, representing a remarkable transformation in the approach to the disease. These new agents include four multitargeted, VEGF-R kinase inhibitors (sorafenib, sunitinib, pazopanib, axitinib), two mTOR inhibitors (temsirolimus and everolimus) as well as monoclonal anti-VEGF antibody (bevacizumab) in combination with IFN. Each of these treatments has shown progression-free survival benefit compared with either IFN, placebo, or another kinase inhibitor in randomized trials [67–77]. Overall survival benefit in these same trials has been difficult to demonstrate due to significant on- or post-study crossover.

A number of large-scale, placebo-controlled, randomized trials have been initiated since 2006 to investigate the adjuvant utility of the new agents (Table 14.5) [78–83]. Five of these trials are studying VEGF-R tyrosine kinase inhibitors (TKIs), while one is investigating mTOR inhibition [84]. In early 2015, initial results became available for the ASSURE trial of sorafenib, sunitinib, or placebo. An interim analysis revealed no significant differences in disease-free or overall survival between either of the experimental arms and placebo. Median disease-free survival was 5.8 years (HR = 1.01, 97.5 % CI 0.83–1.23) for sunitinib, 5.8 years (HR = 0.98, 97.5 % CI 0.81–1.19) for sorafenib, and 6.0 years for placebo. While these results are disappointing, longer follow-up for mature overall survival data is needed, and the results of the remaining adjuvant studies remain eagerly anticipated. At the current time, observation remains the standard of care for managing postsurgical patients, and placebo control of ongoing adjuvant studies remains ethically valid.

Several factors make VEGF-R tyrosine kinase inhibitors (TKIs) and mTOR inhibitors attractive for use in the adjuvant setting. Foremost, the drugs have proven activity against metastatic RCC with frequent tumor regression and the ability to stabilize disease and delay progression. The oral availability of most of these agents makes them well suited for adjuvant use. While side

Table 14.5 Ongoing second-generation adjuvant trials

Experimental arm	Control arm	Name	Sponsor	N	Risk category or stage	End point	Accrual years
Sorafenib 1 year or Sunitinib 1 year	Placebo	ASSURE (E2805) [78]	ECOG	1,923	Intermediate–high risk (UISS)	DFS	2006–2010
Sunitinib 1 year	Placebo	S-TRAC [79]	Pfizer	720	High risk (modified UISS)	DFS	2007–current
Sorafenib 1 year or sorafenib 3 years	Placebo	SORCE [80]	MRC	1,656	Intermediate–high risk (Leibovich)	DFS	2007–2013
Pazopanib 1 year	Placebo	PROTECT [81]	GSK	1,500	T2 N0 G3–4 or T3–4 N0 or N1 (2010)	DFS	2010–2013
Everolimus 1 year	Placebo	EVEREST (S0931) [82]	SWOG	1,170	Intermediate–high risk (UISS)	RFS	2011–current
Axitinib 3 years	Placebo	ATLAS [83]	SFJ/Pfizer	592	T2–4 N0 or N1	DFS	2012–current

effects including skin reactions, diarrhea, and stomatitis can hinder therapy, these adverse reactions can most often be minimized through supportive care and dose interruption. However, patients' acceptability of side effects in the adjuvant setting may be less, and several of the current adjuvant studies have had unexpectedly high dropout rates [85] due to adverse events. Additionally, the significant activity of these drugs against metastatic disease does not guarantee effectiveness in the adjuvant setting. The very infrequent incidence of complete responses with targeted agents along with their tendency to induce disease stabilization as opposed to regression raises question as to the whether these agents can eradicate micrometastatic disease.

Optimal duration of adjuvant therapy may develop as a question as data with targeted agents continues to emerge. With cytotoxic chemotherapy, obtaining total cell kill of micrometastatic disease with cyclical administration of chemotherapy over a defined period is a rationale and effective strategy in certain cancers [86]. As VEGF-R TKIs and mTOR inhibitors are thought to have a predominantly antiangiogenic and growth inhibitory effect as opposed to a direct cytotoxic effect, continued therapy in the adjuvant setting may be needed in order to prevent relapse. This is evidenced in metastatic disease where withdrawal of the agent usually results in subsequent disease progression. Only one of the current adjuvant trials, the UK Medical Research Council's SORCE trial, is addressing the role of duration with the two experimental arms evaluating different lengths of sorafenib therapy (1 and 3 years) [80]. The remaining trials are investigating an empiric 1 year of treatment, with the exception of the ATLAS trial of axitinib, which has a 3-year treatment duration [83].

Appropriate lessons may be learned from use of noncytotoxic systemic adjuvants in other diseases, including hormonal therapy in breast cancer and imatinib in gastrointestinal stromal tumor (GIST). Despite a number of studies addressing the question of duration in breast cancer, the optimal length of adjuvant hormonal therapy is not known. However, studies have suggested that longer durations are more effective than shorter durations [87]. Three years of imatinib was found to be superior to 1 year in the adjuvant setting for GIST [88]. These findings may be relevant to guide adjuvant RCC therapy given the use of TKIs in both diseases. If it is eventually learned that long-term therapy with a targeted agent is necessary for optimal adjuvant effect in RCC, it will be vital to improve prognostication in order to select those patients at appropriate risk who warrant chronic therapy with its associated side effects.

The ongoing trials studying VEGF-R TKIs and mTOR inhibitors represent the largest adjuvant trials in RCC conducted to date. The number of ongoing studies and their rapid accrual are testament to the enthusiasm of urologists and oncologists in finding effective adjuvant therapy for RCC. The large sample sizes and placebo-controlled design of second-generation trials will

result in data that are more robust than previous. With the results of these trials beginning to emerge, we must be considering the questions to ask in future studies. Issues such as duration of therapy and appropriate control arms will arise. Improving patient selection for adjuvant therapy will remain an ongoing challenge. Finally, the development of molecular biomarkers that can both improve risk stratification and predict benefit from specific targeted therapies is greatly needed and is the path to truly personalized adjuvant therapy for RCC.

Conclusions

The unpredictable nature of RCC can be partially mitigated by the use of staging and prognostic systems to determine the risk of relapse after nephrectomy. The range of therapies that have been tested as adjuvants to nephrectomy is remarkable and reflects the historical elusiveness of effective systemic treatments for this disease. Despite many adjuvant trials, no therapy has yet been shown to improve outcome compared to surgery alone. Autologous vaccines have suggested some benefit, but methodology issues cloud the data. The development of effective VEGF and mTOR-directed drugs for metastatic RCC has renewed interest in finding useful adjuvant therapies for this disease. A number of large, placebo-controlled trials are currently being conducted to test the ability of these drugs to delay or prevent disease relapse in the post-nephrectomy setting. Results of these trials are eagerly awaited, and if positive results are seen, the paradigm of localized RCC management will change.

Clinical Vignette

A 60-year-old man with a history of hypertension and hypercholesterolemia presented with hematuria. He described fatigue developing over several months. His ECOG performance status was 1. Workup included a CT scan that revealed a large mass arising from the upper pole of the left kidney. Several left periaortic regional lymph nodes measured up to 2.4 cm. No evidence of distant metastatic disease was seen on imaging. He underwent left radical nephrectomy with a periaortic lymph node dissection. Pathology revealed a 10.5 cm clear cell renal carcinoma, Fuhrman grade 2 with focal penetration into the perirenal fat, and no noted necrosis. Two of 12 dissected nodes were involved with carcinoma. The tumor was stage III (T3aN1M0) by the 2010 edition of the TNM staging system. His prognostic score was 6 by the SSIGN system and category III by UISS. TNM, SSIGN, and UISS estimates of 5-year survival were 53 %, 54 % (cancer specific), and 39 %, respectively.

The risk of disease recurrence was explained to the patient. It was explained that there are currently no adjuvant treatments that have proven effective in improving his chance of survival from renal carcinoma. The patient was offered enrollment in a placebo-controlled, phase III trial of adjuvant everolimus. He consented for the study and met eligibility criteria. Treatment with blinded study drug was associated with moderate fatigue and stomatitis. He completed a year of study treatment. Eighteen months after nephrectomy, CT imaging revealed no evidence of recurrence or distant metastases. He continues on a surveillance regimen with regular imaging to monitor for disease recurrence.

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Cytokines in the Management of Advanced Renal Cell Cancer

15

Radha Verman and Primo N. Lara Jr.

Contents

Key Points

- IFN-α has modest activity in metastatic renal cell cancer (mRCC). Currently, its main therapeutic role is in combination with bevacizumab, which has been approved for first-line therapy.
- High-dose IL-2 can lead to durable responses not seen with any other drug, but should be considered as first-line therapy only for highly selected favorable or intermediate-risk patients due to its severe systemic toxicities.
- Proper management of adverse events due to high-dose IL-2 can limit toxicity and improve patient outcomes.
- Combinations of immunotherapy and cytotoxic chemotherapy are not effective and therefore not recommended for current treatment of mRCC.
- Combination of immunotherapy and biologic agents is of limited use due to increased toxicity, with the exception of IFN and bevacizumab, which appears to be both tolerable and efficacious.
- Efforts are underway to elucidate molecular markers that will help predict benefit from the administration of high-dose IL-2.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_15

Table 15.1 Selected immune-based approaches

Interferons (*INF*): interferon-α, interferon-β, interferon-γ
Interleukins (*IL*): interleukin-2, interleukin-12, interleukin-21
Cytokine combination strategies
Cytokine combinations
Cytokines + cellular therapies (e.g., IL-2 and tumor-infiltrating lymphocytes)
Cytokines and chemotherapy or biologics (e.g., INF + bevacizumab)
Mini-allogeneic transplant approach
Reduced-intensity conditioning therapy followed by circulating hematopoietic progenitor cell transplantation
Tumor vaccines
Tumor cell-based vaccines
Gene-modified tumor cell vaccines
Dendritic cell-based vaccines
Heat shock protein-based vaccine
Antigenic peptide-based vaccines
Immune checkpoint inhibition
Anti-PD1 monoclonal antibodies (nivolumab)
Inhibition of cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)

15.1 Overview

The hypothesis that renal cell cancer (RCC) may be sensitive to immunologic manipulation initially came from the rare but fascinating phenomenon of spontaneous tumor regression in RCC patients [1]. The mechanism for spontaneous regression is unclear, although immunologic factors have been implicated. This has led to the evaluation of immune-based strategies in the management of advanced disease. Table 15.1 provides a summary of selected immune-based approaches [2] that have been employed in RCC; however, this chapter will focus predominantly on cytokine-based therapies (see Chaps. 14 and 16 for a discussion of other immune-based therapies, including vaccines and checkpoint inhibitors).

Cytokines are non-antibody proteins used for cellular communication and can act as mediators or regulators of the immune system. Some of the most studied cytokines include interferon alpha (IFN-α) and interleukin-2 (IL-2). These cytokines have long been considered important factors in the activation and development of an immune response, including responses against tumor cells. These responses are believed to be mediated through enhanced T-cell, dendritic cell, and natural killer (NK)-cell activity directed against antigenic RCC cells. The discovery of methods to manufacture and purify cytokines through recombinant technology triggered a series of trials testing these agents in patients with advanced RCC.

15.2 Interferon

IFN-α is a cytokine that stimulates cytolytic activity and proliferation of NK cells, phagocytic functions and production of other cytokines by macrophages, and the expression of MHC molecules in most immune cells [3]. Another mechanism by which IFN-α operates is through regulation and proliferation of cytotoxic CD8+ T cells [4]. It is thought that IFN-α stimulates the proliferation, activation, and generation of CD8+ T cells leading to tumor cell destruction. In cancer, there is also dysregulation observed between T-helper (Th) 1 and Th2 CD4+cells, characterized by an imbalance in Th2 CD4+ cell production [5]. Th1 CD4+ cells mature to become macrophage-activating cells, whereas Th2 CD4+ cells turn into B cells. IFN-α can stimulate the expression of IL-12 receptors on Th1 cells leading to selective promotion of the Th1 response and also causing a suppression of IL-4 and IL-13 gene expression. This culminates in a subsequent dampening of the Th2 response [6]. This series of events is believed to lead to an enhancement in the activity of the cellular immune response wherein monocytes and macrophages exert a direct negative effect on tumor cell growth and proliferation via their phagocytic mechanisms. IFN-α also exerts its antitumor activity through its ability to upregulate MHC gene expression in tumor cells. Most tumor cells exhibit a partial or complete loss of MHC antigens on the cell surface [7]. This does not allow for dendritic cells – antigen-presenting cells (APCs) that are potent stimulators of IFN-α production – to recognize

nonself antigens and to initiate the cytokine cascade. This can then lead to an indirect enhancement of the proliferation of tumor cells. Antitumor therapies that upregulate MHC gene expression in tumor cells, such as IFN-α, are thought to induce immunologic rejection of the tumor cells through the activation of APCs and cell-mediated cytotoxicity.

Three categories of interferons of relevance to RCC have been described: IFN-α, IFN-β, and IFN-γ. These IFN species vary according to the usual cell of derivation. IFN-α is mainly derived from white blood cells and IFN-β from fibroblasts, while IFN-γ is typically derived from T cells. As noted earlier, recombinant technology has allowed for the efficient manufacture of these molecules for human testing in clinical trials. The most active agent appears to be IFN-α, while IFN-β and IFN-γ appear to be of limited clinical utility. For example, in a phase II trial single-agent IFN-β serine in RCC, there was no signal of enhanced efficacy for IFN-β serine compared to historical data with IFN-α [8]. Furthermore, a placebo-controlled trial in metastatic RCC of IFN-γ 1b (dosed at 60 μg per square meter of body surface area subcutaneously once weekly) showed no significant differences between the groups in terms of response rates, time to disease progression, or overall survival. Thus, further clinical development of IFN-β and IFN-γ had been halted, while IFN-α was subsequently evaluated in a series of clinical trials.

Recently, there has been revival of interest in IFN-γ research. A study by Chen et al. draws attention to two issues limiting IFN-γ efficacy, which include previously exploiting only its immunomudulatory properties rather than its direct tumoricidal properties and its poor pharmacokinetics, which was improved by developing an antibody-cytokine conjugate. In this in vitro study, the investigators demonstrate that both human and murine IFN-y fused to an anti-CD70 antibody are able to induce RIP1-dependent necrosis in RCC cells in the presence of the proteasome inhibitor bortezomib [9]. Further studies evaluating IFN-γ are ongoing.

Wide ranges of dosing regimens and schedules for IFN-α have been employed across clinical trials. At this time, no one dose schedule has been definitively identified as the most optimal. A regimen of nine million units given by subcutaneous injection, three times a week for 12 weeks or to disease progression, has been widely used in the control arms of recently completed randomized phase III trials [10–14]. In 1990, IFN-α was approved for the treatment of metastatic renal cell carcinoma in Western Europe based on nonrandomized phase II studies. Notably, IFN-α has never received US Food and Drug Administration (FDA) approval for its use in advanced RCC (Fig. 15.1 shows the proposed 3D structure for the recombinant IFN-α2b molecule as depicted in RCSB Protein Data Bank at http://www.rcsb.org).

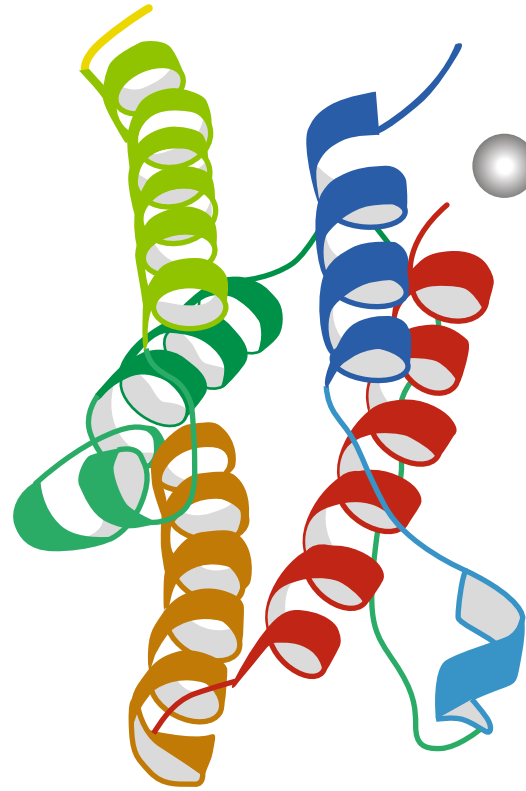

Fig. 15.1 Proposed three-dimensional structure of recombinant interferon alpha-2b (http://www.rcsb.org)

A number of randomized phase III studies have been completed using IFN-α in the setting of metastatic RCC; it must be noted that none of the trials were placebo controlled. One study

compared IFN-α2b with medroxyprogesterone acetate (MPA) [15, 16]. Patients with mRCC were randomized to receive either subcutaneous IFN-α2b (three doses, five million units, five million units, and ten million units for the first week, and then ten million units three times per week for a further 11 weeks, with a total number of patients = 174) or oral MPA (300 mg once daily for 12 weeks, with a total number of patients = 176). A total of 111 patients died in the IFN-α2b group compared to 125 patients in the MPA group. There was a relative reduction in the risk of death by 28 % in the IFN-α group (hazard ratio 0.72 [95 % CI 0·55–0·94], $p = 0 \cdot 017$). IFN-α2b gave an absolute improvement in 1-year survival of 12 % (MPA 31 % survival vs. IFN-α2b 43 %) and an improvement in median survival of 2.5 months (MPA 6 months vs. IFN-α2b 8.5 months). Side effects were more common with the IFN-α2b group and included moderate to severe lack of appetite, nausea, lack of energy, shivering, and dry mouth. Other studies compared IFN-α2a plus vinblastine with either vinblastine alone [16–18] or against MPA [19]. When IFN-α and vinblastine were compared to vinblastine alone, the interferon-containing arm was superior in terms of response rates (17 % vs. 3 %) and survival (67.6 vs. 37.8 weeks, $p < 0.05$). On the other hand, when the combination IFN-α2a and MPA was compared to MPA alone, there was a significant difference in response rate (21 % vs. 0 %), but not in overall survival (16 months vs. 10 months, $p = 0.19$).

This notion was confirmed in a 2005 Cochrane review of published randomized controlled trials employing IFN-α in advanced RCC [20]. Pooled results from four trials consisting of 644 patients suggested that IFN-α was superior to controls (odds ratio for death at 1 year was 0.56, 95 % CI 0.40–0.77), while the overall hazard ratio for death was 0.74 (95 % CI, 0.63–0.88). The pooled remission rate was 12.5 % for IFN-α versus 1.5 % for controls, with a pooled odds ratio of 7.6 (95 % CI 3.0–19.2). The weighted average improvement in survival was 3.8 months (11.4 vs. 7.6 months). Based on these results, IFN-α became a reasonable community standard for the systemic management of advanced RCC. Recently, the discovery of novel targeted agents has decreased the use of IFN-α with its application limited to combination therapy with biologic agents (discussed later in this chapter and in Chap. 17).

Observational case reports noted improved responses and survival when the primary tumor was removed surgically. This was the impetus for a randomized trial comparing IFN-α to nephrectomy followed by IFN-α in mRCC conducted by the Southwest Oncology Group (SWOG trial 8949). The results were noteworthy for a significant improvement in median overall survival in patients who had a nephrectomy prior to immunotherapy. The median overall survival in the group receiving IFN-α only was 8.1 months, while the median overall survival in the group of patients who received a nephrectomy followed by IFN-α was 11.1 months [21]. An updated analysis with a median follow-up of 9 years was conducted to evaluate predictors of overall survival. Patients randomized to nephrectomy continued to demonstrate improved overall survival (HR 0.74, 95 % CI 0.57–0.96, $p = 0.022$). Multivariate analysis showed that performance status 1 vs. 0 (HR 1.95, $p < 0.0001$), high alkaline phosphatase (HR 1.5, $p = 0.002$), and lung metastasis only (HR 0.73, $p = 0.028$) were overall survival predictors [22]. The findings seen in the SWOG 8949 were confirmed by another similar but much smaller randomized trial conducted by the European Organization for Research and Treatment of Cancer Genitourinary Group (EORTC 30947). This trial reported a significant increase in the time to progression (5 months vs. 3 months) and median survival duration (17 months vs. 7 months) in the group that underwent debulking nephrectomy followed by IFN-α when compared to IFN-α alone [23]. Furthermore, when both of these trials were combined in a meta-analysis conducted by the Cancer Care Ontario Program in Evidence-Based Care (CCO-PEBC), the overall median survival time for patients treated with nephrectomy and INF-α2b was 13.6 months compared with 7.8 months for patients treated with INF-α2b alone ($p = 0.002$). This represents a 31 % decrease in the risk of death in the surgical arm [24].

These data support the role for cytoreductive nephrectomy. Among the many caveats here are that some patients who undergo surgery may have resultant complications that either delay or make them ineligible to receive further systemic therapy. Nevertheless, IFN-α following debulking nephrectomy in patients fit enough to undergo the procedure should be considered as part of the standard treatment strategy in mRCC.

15.3 Interleukin-2

Interleukin-2 is an immune cytokine that is essential in the activation of a specific response to antigens by T cells, as well as crucial in triggering the innate immunity by stimulating several functions of NK cells and macrophages [25]. The actual mechanism by which IL-2 exerts its antitumor effects is unknown, but there are several hypotheses. Experiments in animal models showed that IL-2 can offset defective antigen recognition and overcome tolerance, thus suggesting its use as therapy to stimulate tumor destruction by T- or NK-cell activation while overcoming possible forms of tolerance or immunological ignorance which are known to occur toward tumor antigens [25]. In vitro studies with murine and human cells showed that IL-2 can activate lymphokine-activated killer (LAK) cells, a subpopulation of lymphocyte effectors that include NK, T, and NKT cells. These cells are endowed with the capacity of killing neoplastic cells in a MHC-unrestricted fashion. Clinical trials have noted a response in the tumor burden of patients treated with IL-2, but the mechanism of such clinical responses has not been clarified since accumulation of LAK cells in metastatic deposits (i.e., direct tumor kill) has not yet been demonstrated [25]. Thus, tumor shrinkage has been attributed to nonspecific cytotoxic activity of LAKs as well as to activation of tumor-specific T cells, but the release of tumor cytotoxic cytokines (e.g., TNF-α) by activated lymphocytes may also have contributed.

A total of 255 patients with metastatic RCC were entered onto seven phase II clinical trials and treated with high-dose IL-2 at either 600,000 or 720,000 international units per kg (IU/Kg) per dose intravenously every 8 h for up to 14 consecutive doses until maximally tolerated [26, 27]. A second identical cycle of treatment was scheduled following 5–9 days of rest. These courses could be repeated every 6–12 weeks in stable or responding patients for a total of three courses. The overall response rate was 14 % with 12 (5 %) complete responses and 24 (9 %) partial responses. The median response duration was 19 months for partial responders and had not been reached for complete responders. The median overall survival was 16.3 months [27]. These studies showed that patients who responded to IL-2 could attain a durable response and were living longer than historical controls that had received no therapy. The durability of response was confirmed elsewhere when 6 % of patients with metastatic renal cell cancer treated with high-dose IL-2 were found to be in complete remission from 4 to 10 years after treatment [28]. Based on the phase II single-arm studies discussed above, the FDA approved the dose of 600,000 IU/kg (high-dose IL-2) in 1992 for the treatment of metastatic RCC as front-line therapy.

High-dose IL-2 is associated with systemic toxicities and can affect every organ system in the body. Patients are generally admitted to an intensive care unit or similarly staffed unit for the administration of this drug. Prior to initiating therapy, one must make sure that the patient does not have significant cardiac, pulmonary, or renal disease. During a typical treatment course, patients will often experience the following symptoms occurring at different time points within the course. Within 2–3 h after the first or second dose of IL-2, patients often start experiencing fevers and chills. Around this same time, patients will also start experiencing mild hypotension and tachycardia that will progressively become more severe with each dose. They will typically establish a new baseline blood pressure around 20–30 mmHg below their usual blood pressure. Oliguria usually starts within the first 24 h, requiring small boluses of fluid to keep urine output greater than 20 ml/h. As the patient nears the end of the cycle, hypotension and

oliguria can become refractory to judicious hydration (no more than 1–1.5 L per day) requiring pharmacologic intervention including dopamine and phenylephrine. Pulmonary congestion, increase in weight, and peripheral edema may then ensue due to fluid overload and as a manifestation of capillary leak. Nausea, vomiting, and diarrhea also occur closer to the completion of the cycle [29]. Neurologic, infectious, metabolic, and dermatologic effects can also be manifested; these are specified in more detail in Table 15.2. These symptoms are primarily thought to be due to capillary leak syndrome (CLS) and lymphoid infiltration within the organ systems. Proper management of the adverse events discussed above can limit toxicity and improve patient outcomes.

Given the difficulty of administering high-dose IL-2, attempts were made to find a lower dose of IL-2 or an alternative administration schedule, whereby its antitumor activity would be preserved with diminished or mitigated side effects. A three-arm study sponsored by the National Cancer Institute compared high-dose IL-2 administered at 720,000 international units/kg to low-dose IL-2 dosed at 72,000

Table 15.2 Side effects and management of high-dose IL-2 administration

System	Adverse reaction	Treatment
Cardiovascular	Hypotension due to capillary leak syndrome	Fluids (normal saline), limit to 1–1.5 L/day
		Add phenylephrine drip if refractory to fluids
	Sinus tachycardia due to hypotension	Increase time between doses of IL-2
	Atrial fibrillation or ventricular arrhythmia	Hold IL-2, evaluate for ventricular damage (ischemia), correct electrolytes and anemia, and use medications as needed. Restart IL-2 only if arrhythmia is easily corrected
	Peripheral edema	Hold IL-2, watchful waiting as this will resolve over time or with the use of diuretics. Elevate extremity
	Increased troponin or creatinine kinase	Hold IL-2; exercise ECHO before next dose of IL-2 to evaluate for myocardial dysfunction. If evidence of ischemia, stop IL-2
Pulmonary	Hypoxia – fluid overload	Diuretics
	Tachypnea – due to hypoxia or metabolic acidosis	Diuretics if due to fluid overload
		IV sodium bicarbonate
Renal	Elevated creatinine with adequate urine output	Fluids (normal saline), limit to 1–1.5 L/day
		Add dopamine drip if unresponsive/unable to tolerate fluids
		If oliguria and/or elevated SCr, hold IL-2
Neurologic	Confusion, disorientation, hallucinations	Hold IL-2 until resolution; then rechallenge. If symptoms are recurrent, then hold treatment
Metabolic	Metabolic acidosis	Bicarbonate infusion (100 meq/L) to keep serum bicarbonate level >18 meq/L
	Hypokalemia	Replace electrolytes as needed
	Hypocalcemia	
	Hypomagnesemia	
Systemic	Fevers and chills	Premedication with acetaminophen 650 mg po q4h and indomethacin 25 mg po q6h. An H2 blocker to protect the gastric mucosa should be utilized. Consider infectious etiology if first fever is over 24 h after therapy initiation
	Rigors	Meperidine 25–50 mg IV × 1
	Nausea and vomiting	Ondansetron 4 mg IV × 1
		Prochlorperazine 25 mg IV × 1
Skin	Dermatitis	Topical emollients and antihistamines. Avoid steroid- or alcohol-containing lotions
	Pruritus	Histamine antagonist (e.g., diphenhydramine)
Gastrointestinal	Diarrhea	Diphenoxylate or loperamide as needed

international units/kg to low-dose subcutaneous daily IL-2 [30]. Response rate was significantly higher with the high-dose compared with the low-dose IV and subcutaneous schedules (21 % vs. 13 % vs. 10 %, respectively). There were more adverse events in the high-dose IV therapy group, but no deaths were attributed to it. There was also a trend toward more durable responses with the high-dose IL-2 group. Overall, there was no difference in overall survival. Toxicities though were seen much less frequently in the low-dose arm, especially the major side effect of hypotension. Although, subcutaneous IL-2 did not have a significant response rate in this study, impressive response rates were seen in patients with metastatic RCC in other phase II trials [31–33]. This led to the popularization of this mode of therapy in European countries in the 1990s. There was however no definitive studies conducted to fully evaluate its utility and its place among the treatment options for metastatic RCC.

More recently, a systematic review evaluating patients with unresectable or mRCC, comparing treatment regimens containing IL-2 to those without, revealed that mortality at 1 year was not statistically significant between IL-2-based regimens and non-IL-2 controls [34]. The pooled response rates, however, were higher in patients receiving IL-2-based regimens (range, 9–39 %) compared with non-IL-2 controls (0–20 %). There was an increase in toxicity in the IL-2-based regimens compared to non-IL-2 controls; however, most patients tolerated treatment well. Of note, this review did not include any high-dose IL-2 trials, as there are no known randomized trials comparing high-dose IL-2 to non-IL-2 control or placebo (all prior studies were phase II single-arm studies).

Based on the data above, non-high-dose IL-2-containing regimens do not appear to provide superior treatment efficacy over non-IL-2-containing regimens and are associated with increased toxicity. High-dose IL-2 does provide higher response rates, albeit with higher toxicity, and can provide a small chance for a complete and durable remission and hence continues to play a role in the treatment of mRCC in the appropriate treatment population.

15.4 Interferon plus Interleukin-2 Combination(s)

Interferon alpha and interleukin-2 have been shown to have efficacy in the treatment of metastatic RCC; however, whether these two drugs given in combination would be more efficacious was the subject of intense investigation in the 1990s.

Phase II trials were first performed to assess combining these two agents in hopes of a synergistic response. One study evaluated high-dose IL-2 alone (1.33 mg/m^2; approx. 600,000 IU/kg) versus non-high-dose IL-2 (0.8 mg/m^2) in combination with IFN-α in patients with mRCC [35]. In this study, patients in both arms had responses to therapy, but the IL-2 alone arm (high-dose IL-2) was noted to have a higher objective and durable response rate. This study concluded that IL-2 alone, when given as a high-dose IV bolus, was active in metastatic RCC and that combining it with IFN-α was not as efficacious. A somewhat varying conclusion was noted from a publication around the same time that had tested alternate daily dosing of intravenous IL-2 and subcutaneous IFN-α [36]. In that study, 36 patients received 14 days of daily alternating treatments of IL-2 and IFN-α every 6 weeks for up to four cycles. Of the 30 patients who completed at least two cycles, there were nine objective responses, and seven of them had relapse-free survival times that were >6 months, the longest being 2 years. The toxicity was reported to be less, and these results led to a conclusion that the combination of IL-2 and IFN-α was active, rivaled responses of each agent alone from other phase I and II studies, and warranted further study. Other phase II studies were carried out in order to evaluate the use of subcutaneous IL-2 and IFN-α [37–39]. These studies noted encouraging responses with less toxicity, but results were discordant and did not provide definitive conclusions.

In this setting, the Groupe Francais d'Immunotherapie initiated one of the first randomized phase III studies that established the efficacy of IFN-α and IL-2 in patients with metastatic RCC in 1998 [40]. Patients were randomized to receive either subcutaneous injections of

IFN-α, continuous intravenous infusion of IL-2, or both given in combination. The dose of IL-2 used in this study was an intermediate one, 18,000,000 IU/m^2 per day (i.e., non-high dose). Response rates were 6.5 %, 7.5 %, and 18.6 % ($p=0.01$) for the groups receiving IL-2, IFN-α, and IL-2 plus IFN-α, respectively. Over a period of 1 year, the event-free survival was 15 %, 12 %, and 20 %, respectively ($p=0.01$). Despite the encouraging results of combined therapy, there was no difference in overall survival between the three groups. The investigators also noted more adverse events in the combined immunotherapy group. Hence, it could not be concluded that combined therapy provided a significant advantage. Another phase III study evaluated the inpatient administration of high-dose IL-2 to the outpatient regimen of subcutaneous IL-2 and IFN-α [41]. The response rate was 23.2 % for high-dose IL-2 versus 9.9 % for IL-2 and IFN-α ($p=0.018$). Ten patients receiving high-dose IL-2 were progression-free at 3 years versus three patients receiving IL-2 and IFN-α ($p=0.082$). These results suggest that high-dose IL-2 is more efficacious when compared to outpatient subcutaneous IL-2 and IFN-α combined.

In summary, there were a variety of combinations of IL-2 and IFN-α that were tested in the 1990s and early 2000s. Overall, the combination appeared to have some efficacy, but randomized phase III trials did not demonstrate an improved survival rate when comparing varying doses of IL-2 combined with IFN-α to that of high-dose IL-2 alone. Hence, high-dose IL-2 alone should remain a standard of care option for highly selected patients with mRCC.

15.5 Cytokines in Combination with Chemotherapy

There were subsequent efforts to improve upon the modest survival advantage seen with IFN-α. However, when combinations with cytotoxic or differentiating drugs were attempted, the results were disappointing. For instance, the differentiating agent 13-cis retinoic acid showed some promise in the treatment of metastatic RCC, but when this drug was combined with IFN-α, the results showed no improvement in survival when compared to monotherapy with IFN-α [42]. Vinblastine was considered to be somewhat promising when phase II studies showed response rates varying from 16 to 39 % [43]. Unfortunately, phase III trials that compared the combination of IFN-α with vinblastine did not show any improvement in overall survival when assessing it against IFN-α alone [17]. When IFN-α and vinblastine were compared to medroxyprogesterone acetate, which is essentially a placebo arm, no difference in overall survival was noted [18]. In that study, the response rate was 20.5 % in the combination therapy arm and 0 % in the control arm. The lack of a significant difference in survival may have been due to the small number of patients in the study (89 patients total), due to an increase in toxicities in the combination therapy arm, or because response rates in this case do not correlate well with overall survival. Similar results were again noted when the combination of IFN-α and vinblastine showed inferior results in a large phase III trial that compared this combination to an arm with subcutaneous IL-2 and subcutaneous IFN-α and 5-fluorouracil or oral 13-cis-retinoic acid [44].

The fluoropyrimidine 5-fluorouracil had been tested in phase II trials in patients with metastatic renal cell cancer, and response rates varied from 12 to 39 % [45, 46]. 5-Fluorouracil looked to be fairly promising when added to immunotherapy; however, a direct phase III comparison between cytokines plus 5-fluorouracil versus immunotherapy alone was required. This was fulfilled with the completion of the phase III MRC RE04/EORTC GU 30012 randomized study [47]. In that trial, 1,006 treatment-naive RCC patients were randomly assigned to receive interferon alpha-2a alone or combination therapy with interferon alpha-2a, interleukin-2, and fluorouracil. The primary endpoint was overall survival. Serious adverse events were comparable between the arms. At a median follow-up time of 37 months, median overall survival time was reported to be 18.8 months for patients receiving interferon alpha-2a versus 18.6 months for those receiving combination therapy. The hazard ratio

for overall survival was 1.05 [95 % CI 0.90–1.21, $p=0.55$], and the absolute difference was 0.3 % (−5.1 to 5.6) at 1 year and 2.7 % (−8.2 to 2.9) at 3 years. This large randomized trial clearly demonstrated that the polypharmacy approach of cytokines plus cytotoxic chemotherapy was no more efficacious than cytokines alone.

15.6 Cytokines in Combination with Biologic Agents

Over the next decade, the emergence of molecular targeted therapy with tyrosine kinase inhibitors (TKIs) and the mammalian target of rapamycin (mTOR) inhibitors supplanted the use of IFN-α and IL-2. These new drugs (including sunitinib and temsirolimus, both of which are discussed in greater detail elsewhere in this textbook) were more efficacious than single-agent IFN-α in randomized studies. Overall, these studies have shown that combined therapy leads to greater toxicity, which limits their use as a chronic treatment option. Unlike the agents discussed above, bevacizumab, a monoclonal antibody that binds to and neutralizes vascular endothelial growth factor (VEGF), appears to be both tolerable and efficacious in combination with IFN.

In the AVOREN trial [9] which was principally conducted in Europe, 649 patients with previously untreated metastatic RCC were randomly assigned to receive bevacizumab (10 mg/kg every 2 weeks) plus IFN-α (nine million international units subcutaneously three times a week; $n=327$) or IFN-α plus placebo ($n=322$). The progression-free survival was found to be 10.2 months with bevacizumab plus IFN-α versus 5.4 months with IFN-α plus placebo, corresponding to a hazard ratio [HR] of 0.63 ($p<0.001$). The overall response rate (ORR) was also improved in the combined therapy arm (30.6 % versus 12.4 %; $p<0.001$). There was a trend toward overall survival (OS) improvement, with the median overall survival time of 23.3 months with bevacizumab plus IFN-α versus 21.3 months with IFN-α plus placebo (unstratified hazard ratio [HR] = 0.91; 95 % CI, 0.76–1.10; $p=0.3360$; stratified HR = 0.86; 95 % CI, 0.72–1.04; $p=0.1291$). The main confounder was that >50 % of patients in both arms received at least one other post-protocol therapy, including very active tyrosine kinase inhibitors. The above findings were confirmed in additional trials discussed below.

The Cancer and Leukemia Group B (CALGB) 90206 trial was an open-label, phase III trial conducted in the United States, comparing bevacizumab plus IFN-α to IFN-α monotherapy in 732 previously untreated mRCC patients [12]. The median PFS was 8.5 months in patients receiving bevacizumab plus IFN-α compared to 5.2 months in patients receiving IFN-α monotherapy (log-rank $p<0.0001$). The ORR was also improved in the combined therapy arm (25.5 % versus 13.1 %, respectively; $p<0.0001$). The median OS was 18.3 months for bevacizumab plus IFN vs. 17.4 months for IFN monotherapy (unstratified log-rank $p=0.097$; stratified HR = 0.86; 95 % CI, 0.73–1.01; stratified log-rank $p=0.069$). OS favored the bevacizumab plus IFN arm; however, it failed to meet significance, which may be due to postprogression therapy, a factor that was not anticipated when the trial was designed [13].

The TORAVA trial was an open-label, phase II trial conducted in France ($n=171$), comparing the combination of bevacizumab (10 mg/kg every 2 weeks) and temsirolimus (25 mg weekly; group A) versus sunitinib (50 mg/day for 4 weeks followed by 2 weeks off; group B) or the combination of IFN-α (9 mIU three times per week) and bevacizumab (10 mg/kg every 2 weeks; group C). The median PFS was 8.2 months (95 % CI 7.0–9.6) in group A, 8.2 months (5.5–11.7) in group B, and 16.8 months (6.0–26.0) in group C. Grade ≥3 AEs were reported in 77, 60, and 70 % of patients in groups A, B, and C, respectively. The authors concluded that the toxicity of temsirolimus and bevacizumab was much higher than anticipated and clinical activity was low compared to the benefit expected from sequential use of each targeted therapy, hence not recommended for first-line treatment in patients with mRCC. The combination of IFN and bevacizumab achieved favorable PFS results [48].

The Bevacizumab and Low-Dose Interferon (BEVLiN) trial was a single-arm, phase II trial

($n = 146$) evaluating the combination of bevacizumab (10 mg/kg every two weeks) and low-dose IFN (3 MIU three times weekly) in patients with untreated mRCC in order to determine if the use of low-dose IFN can maintain clinical benefit while reducing toxicity. The median PFS and OS were 15.3 months (95 % CI, 11.7–18) and 30.7 months (95 % CI, 25.7 – not reached), respectively. The overall response rate (ORR) was 28.8 % (95 % CI 21.4–37.1). Any-grade and grade ≥3 IFN-associated adverse events occurred in 53.4 % and 10.3 % of patients, respectively, and were lower by 17 % and 18 %, respectively, compared with the AVOREN subgroup. The authors concluded that compared with the historical control AVOREN subgroup, low-dose IFN with bevacizumab resulted in a more favorable safety profile, with similar efficacy [49].

15.7 Predictive Clinical Features and Biomarkers for the Use of Cytokines to Treat mRCC

There are a multitude of different agents now available for the treatment of mRCC, yet there are only limited data on how best to determine which patient population cytokines will be most effective, especially given the low response rates and substantial side effects of such therapies. Recent advances in the understanding of the molecular mechanisms underlying RCC are vital for establishing the optimal treatment strategies in patients with mRCC with a drive toward personalized medicine. Here, selected results of recent research into potential biomarkers related to cytokines are discussed.

Retrospective studies evaluated clinical features and/or molecular markers to assess if these could be used to predict response to therapy. Clinical features that were identified included clear cell histology [50] as well as a favorable score on the UCLA Survival after Nephrectomy and Immunotherapy (SANI) scale [51]. The SANI score was developed as an algorithm capable of predicting survival in patients with metastatic RCC who underwent nephrectomy and received IL-2-based immunotherapy. The primary endpoint was survival and was assessed based on clinical, surgical, and pathological features. The multivariate analysis showed that the presence of lymph node involvement, constitutional symptoms, multiple metastatic sites (as compared to bone- or lung-only metastases), sarcomatoid histology, and elevated TSH level had adverse effects on survival.

Upton et al. examined the specimens from patients with RCC treated with IL-2 to identify histologic features that predict response. They found that for clear cell carcinomas, response to IL-2 was associated with the presence of alveolar features and the absence of papillary and granular features [50].

In addition to clear cell histology and the SANI score, the enzyme carbonic anhydrase-IX (CA-IX) has been identified as a potential biomarker to predict outcomes in patients with RCC. It was found to be expressed in 94 % of clear cell RCC tumors but absent in most normal tissue. Low CA-IX staining (<85 %) of tissue microarrays by immunohistochemistry was a poor prognostic factor for survival for patients with mRCC, with a hazard ratio of 3.10 ($p < 0.001$) [52]. A subsequent case-control study by Atkins et al. showed an association between higher levels of CA-IX expression and response to IL-2. The response to IL-2 was further improved in those patients with high CA-IX expression level and histologic predictors based on the Upton pathology model [53]. There was an attempt to prospectively validate these features in a clinical trial of patients with mRCC treated with high-dose IL-2. Preliminary results of this study (SELECT trial) showed that clear cell histology might be the salient clinical feature that selects patients who respond to IL-2 [54]. Unfortunately, it failed to show the predictive capacity of either the CA-IX expression or the favorable histologic features as reported in prior studies.

Recently, data were presented evaluating PDL1 or PDL3 (programmed death ligand 1 or 3) expression and their association with response to initial therapy with IL-2 or subsequent therapy (VEGFR TKI). In the 17 patients whose tumors were positive for both PDL1 and PDL3, the

overall response rate (ORR) to IL-2 was 52.9 %. In the 27 patients that were negative for PDL1 and PDL3, the ORR to IL-2 was 11.1 %. With regard to subsequent VEGFR TKI therapy, those patients whose tumors were positive for PDL1 and PDL3 expression had a shorter duration of VEGFR TKI therapy compared to those that were negative (9.0 months vs. 42.5 months, respectively) [55].

The treatment of mRCC with both IL-2 and IFN-α relies on the ability to activate CD4+ and CD8+ T cells. B7-H4 is a B7 member identified as an inhibitory modulator of the T-cell response, and the upregulation of this ligand is thought to lead to immune escape in mRCC. Krambeck et al. have shown that aberrant RCC expression of B7-H1 leads to disease progression and decreased survival. Furthermore, those tumors expressing both B7-H1 and B7-H4 are at an even greater risk of death from RCC. Because it appears that both of these ligands impair T-cell function, this group infers that they may be useful in determining which patients may respond to IL-2 therapy [56]. Xu et al. confirmed these findings where B7-H4 expression was seen in 59 % of tumor specimens collected from RCC patients undergoing radical nephrectomy. Exposure of a clear cell RCC (ccRCC) cell line to IL-2, IFN-α, and IFN-γ leads to increased expression of both protein and mRNA of B7-H4 and was most apparent after exposure to IFN-γ. Masking of B7-H4 with a specific blocking antibody increased the T-cell-mediated killing of the ccRCC cells. These observations may present evidence for the role of B7-H4 in tumor immune escape in mRCC and may be the reason for the low efficacy of IL-2 and IFN-α and inability to observe efficacy of IFN-γ. In addition, B7-H4 may be further studied as a potential biomarker [57].

Although single-agent IFN-α is rarely used in many resource-rich nations, its use continues in many parts of the world. Due to the low response rate seen with single-agent IFN-α, identification of potential predictive markers of response is necessary to determine which subset of patients will benefit from this drug. Recently, Eto et al. has analyzed a large number of genomic polymorphisms from DNA extracted from whole blood of RCC patients. In an initial retrospective study, they evaluated 463 SNPs on 33 candidate genes and found that SNPs in the signal transducer and activator of transcription 3 gene (STAT3) were associated with a better response to IFN-α in patients with mRCC. In a follow-up trial, these investigators evaluated the correlation between the antitumor effects of IFN-α and 11 SNPs. Overall response (CR and PR) to IFN-α was found not to be associated with any of the 11 SNPs (including STAT3). However, when assessing the clinical response defined as CR, PR, and stable disease of >24 weeks, a significant association was observed between the STAT3-2 and clinical response to IFN-α ($p=0.039$). Furthermore, the C/C genotype of STAT3-2 was associated with the clinical response of IFN-α and the secondary endpoint of overall survival. Note that this study was completed in the Japanese population only and generalization of results to other races/ethnicities is uncertain [58].

At this time, clear clinical predictive factors or molecular biomarkers for the benefit of IL-2 or IFN-α remain elusive and are not yet ready to adopt into clinical practice, but are the focus of ongoing research.

Clinical Vignette

A 50-year-old male with no past medical history noted a cough that has been troubling him for the last 4 weeks. He tried a number of over-the-counter cough suppressants with only minimal improvement in his symptoms. His primary care physician ordered a chest radiograph that revealed numerous lung nodules, the largest being 2×2 cm in the left lower lobe. Follow-up CT scans of his chest, abdomen, and pelvis were then performed and confirmed the lung nodules as well as a 7 cm mass in his right kidney. A biopsy of the left lower lobe lung nodule was performed, and the pathology was consistent with carcinoma with clear cells, establishing the diagnosis of

metastatic renal cell carcinoma. He underwent a cytoreductive nephrectomy, confirming the diagnosis of renal cell cancer. This patient is otherwise healthy and asymptomatic; he runs 3 miles a day and is taking no medications. Although there are many therapeutic choices, high-dose intravenous interleukin-2 should be strongly considered for this young, healthy patient with limited metastatic disease confined to the lungs, as there is potential for a durable complete response. In the 1990s, the mainstay of therapy for metastatic renal cell carcinoma included the use of cytokine agents. Even with the discovery of potent, efficacious, and less toxic biologic agents, there is still a limited role for the use of cytokines today. This chapter will discuss the history and past achievements of cytokine-based immunotherapy as well as the future of these agents.

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Immune Checkpoint Inhibition in Renal Cell Carcinoma

16

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Contents

Research Support Research supported by Claudia Adams Barr Program for Innovative Cancer Research, the 2014 AACR Basic Cancer Research Fellowship, Grant Number 14-40-01-MAHO, and the ASCO Young Investigator Award supported by Kidney Cancer Association (KM).

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_16

Key Points

- The anti-PD-1 blocking antibodies, nivolumab and pembrolizumab, and the anti-PD-L1 blocking antibodies, BMS-936559, MPDL3280A, and MEDI4736, have shown impressive antitumor effects in several malignancies including renal cell carcinoma (RCC), melanoma, Hodgkin lymphoma, non-small cell lung cancer, and urothelial carcinoma.
- Higher PD-L1 expression on both tumor cells and the immune infiltrate are negative prognostic markers for patients with RCC.
- Tumor expression of PD-L1 appears to predict a higher chance of response to PD-1 pathway blockade but as of yet is inadequate as a definitive predictive biomarker for response. Multiple assays have been described, all of which have generally poor sensitivity and specificity, i.e., not all patients with PD-L1-positive tumors respond and tumors expressing little or no PD-L1 can respond, although with less frequency than PD-L1 "high" tumors. Limitations to the use of PD-L1 expression as a biomarker development include the lack of a uniform assay as well as the lack of agreement as to what is scored as "positive."
- While approximately 20–30 % of patients respond to single-agent blockade, phase I trials combining CTLA-4 and PD-1 blockade have shown the potential to significantly improve response rates in both RCC and melanoma.
- Combinations of PD-1 pathway blockade and VEGF-targeted therapeutics, such as bevacizumab or the tyrosine kinase inhibitors (TKI), are under investigation; early data suggest that such combinations are generally tolerable and may have additive activity.
- The kinetics of an antitumor response to immune checkpoint inhibitors may differ from the standard chemotherapy and/or targeted therapies. Standard measures, such as RECIST criteria, may not capture all patients who will benefit from these agents. Determining the optimal clinical endpoint for clinical trials of immune checkpoint inhibitors is critical.
- Toxicity of the PD-1 pathway-blocking agents is generally lower than that observed with high-dose IL-2 or with antibodies that block CTLA-4. However, immune-related adverse events (IrAEs) like pneumonitis can be severe and potentially life-threatening. The majority of these events can be managed or reversed with appropriate management. Education regarding the recognition and management of IrAEs will be essential for maximizing the clinical benefit of immune checkpoint-blocking antibodies, especially as their routine clinical use becomes more widespread.

16.1 Introduction

For decades, there has been intense interest in stimulating an antitumor immune response to treat solid tumors; however, the efficacy of many of the agents studied has been relatively modest and limited to a small subset of patients. RCC is an exception in that it is sensitive to both IFN-α and interleukin-2 (IL-2), although objective response rates hough objective response rates are generally low. Cytotoxic chemotherapy is generally ineffective in RCC. Currently, the standard of care for treatment centers around the molecularly targeted therapies, including agents that inhibit the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways. These agents induce a greater degree of benefit in terms of objective response rate, progression-free survival (PFS), and, when given in sequence, overall survival (OS) to a much broader patient population than the more toxic cytokine-based immunotherapies [1–12]. Despite their broad efficacy, these agents almost never induce complete responses. Thus, there

remains an ongoing role for high-dose IL-2 as the only approved agent capable of inducing durable complete responses in metastatic RCC albeit in less than 10 % of patients [13, 14]. With the discovery of immune checkpoint molecules, which are a series of cell surface proteins that inhibit T cell responses in cancer and chronic infection, we have seen a renewal of interest in engaging the immune system to fight the cancer as well as the unveiling of potential mechanisms by which tumors may evade the immune system. One such mechanism involves the expression of the T cell inhibitory ligand PD-L1 (programmed death-ligand 1) on the cell surface of tumor cells and infiltrating immune cells. When PD-L1 binds to its receptor PD-1 (programmed death-1) on tumor-infiltrating lymphocytes, the PDL1/PD-1 interaction inhibits T cell activation and effector function [15, 16]. Agents that block this interaction are under investigation in many tumor types, including RCC. These drugs have shown promising results both as monotherapies and in combination with standard therapies. In this review, we will examine the early results of immune checkpoint inhibition in RCC, with a focus on clinical studies of PD-1/PD-L1 and CTLA-4 blockade. We will further discuss how these agents may be incorporated into the management of metastatic RCC once FDA approval is obtained.

16.2 Proof of Principle: Immunotherapy with Cytokine Therapy

While an antitumor effect of IFN-α has been described in RCC, melanoma, and a series of hematologic malignancies, the precise mechanism by which this cytokine modulates the immune system is unclear. Historically, IFN-α became the standard of care treatment for RCC after several studies demonstrated increased overall survival as compared to hormonal treatment or chemotherapy [17–20]. However, the side effects of IFN-α are significant and generally diminish a patient's quality of life. Further, complete and durable responses in patients rarely occur. With the development of VEGF-tyrosine kinase and mTOR inhibitors, IFN-α is used less frequently but remains a standard treatment when administered in combination with bevacizumab [10, 21].

Interleukin-2 (IL-2, Proleukin) received FDA approval for the treatment of patients with metastatic RCC in 1992. Approval was based on its ability to induce durable antitumor responses in a small minority of patients, which were largely restricted to a high-dose regimen (HD-IL-2) [13, 14, 22]. With high-dose regimens, objective responses were seen in approximately 15–25 % of patients with advanced RCC. Despite significant on-therapy toxicity, side effects tend to be reversible and short-lived but can be life-threatening [23]. Given that significant clinical experience is required to safely treat patients with HD IL-2, access is generally limited to cancer centers with experience administering this therapy and which have access to ICU-level care. However, the morbidity and mortality agent associated with HD-IL-2 has been significantly reduced through the use of prophylactic antibiotics, cardiac monitoring, and more stringent patient selection [24].

Thus, HD-IL-2 continues to play an important role as a first-line therapy in a highly selected group of patients fit enough to tolerate therapy [25, 26]. Responses can be seen in up to 30 % of patients [14], and over 50 % of complete responders show long-term disease-free survival and are essentially "cured" [13]. Patients whose duration of response lasts over 30 months have a high chance of cure. Given the toxicity of HD-IL-2, and the fact that the majority of patients do not achieve clinical benefit, a great deal of effort has focused on predictive models for determining which patients are the most likely to respond. In the prospective IL-2 SELECT trial, retrospectively identified predictive biomarkers such as clear cell histology, high CA-IX staining, and MSKCC risk status were not predictive of response, with the exception of clear cell histology [14]. Surprisingly, the results of this trial also showed that benefit was not limited to low-risk patients, as the ORR was not significantly different between patients with "good-risk" vs. "poor-risk" by ISM classification (23 % and 30 %; $P = 0.39$) respectively.

The primary mechanism by which IL-2 exerts its antitumor affect is by stimulating an antitumor T lymphocyte response [27]. Activated T cells express the receptor for IL-2, and IL-2 is required for long-term T cell survival. Not surprisingly, studies in patients with melanoma suggest that response to IL-2 is associated with a T_H1 cytokine expression pattern in TIL, marked most prominently by the expression of the T_H1 effector cytokine IFN-γ [28]. More recent data reinforce the notion that IL-2 is a pleiotropic cytokine. Although it clearly can activate CD8+ effector T cells and some NK (natural killer) cells, IL-2 also expands T regulatory cells (Tregs), a set of CD4 T cells that function to dampen an antitumor immune response. Tregs express the transcription factor Foxp3 as well as high levels of the IL-2 receptor alpha chain (CD25) and inhibit antitumor immune responses via multiple mechanisms, including the secretion of suppressive cytokines like IL-10 and TGF-β [29].

16.3 T Cell Co-stimulatory and Co-inhibitory Pathways

The specificity of the effector T cell relies on the two-step activation of naive T cells [30]. The first recognition step involves binding of the T cell receptor (TCR) to a specific cognate antigen presented in the binding groove of a major histocompatibility (MHC) molecule. This recognition step is known as signal 1. Full T cell activation involves engagement of a second receptor on the surface of the T cell (CD28) [31] by one of its ligands (B7-1 or B7-2) [32] on the surface of an antigen-presenting cell. This second recognition step is known as signal 2. Both signal 1 and signal 2 are required to induce T cell proliferation and IL-2 production. In addition to co-stimulatory molecules like CD28, a series of "co-inhibitory" molecules expressed on the surface of T cells has also been identified. The first of these was cytotoxic T lymphocyte antigen-4 (CTLA-4) [33]. When CTLA-4 is expressed on a T cell, it binds tightly to B7-1 on an antigen-presenting cell, essentially hijacking signal 2 and preventing full T cell activation.

Programmed cell death-1 (PD-1) is a second co-inhibitory molecule with a major role in attenuating T cell activation. This member of the CD28/B7 superfamily of receptors was originally isolated from a T cell hybridoma undergoing activation-induced cell death, hence, its name [34]. It was later revealed that PD-1 does not directly engage a cell death pathway. Instead, the interaction between PD-1 and its ligands (PD-L1 and PD-L2) downregulates T cell activation, leading to reduced proliferation and decreased cytokine production [35–37]. Interestingly, the in vitro effects of PD-1 blockade are relatively minor and are limited to modest increases in effector cytokine secretion. So, it seems likely that the impressive in vivo activity of PD-1-blocking antibodies may depend on additional effects on T cell motility, metabolism, and the interaction with antigen-presenting cells [38–40].

16.4 Cytotoxic T Lymphocyte Antigen-4 (CTLA-4)

CTLA-4 is a potent co-inhibitor of lymphocytes with tenfold higher affinity for B7-1 than CD28 (Table 16.1) [41]. The importance of this molecule in T cell homeostasis is clearly illustrated by the lethal phenotype observed in CTLA-4 knockout mice, which succumb to death within 3–4 weeks after birth from massive lymphocyte tissue infiltration and organ failure [42]. Thus, CTLA-4 appears to be involved in early T lymphocyte tolerance. On activated lymphocytes, CTLA-4 is induced and expressed on the surface. When it binds to its ligands, it suppresses the T cell response and downregulates CD28 by facilitating endocytosis [43]. CTLA-4 is highly expressed on T regulatory cells (Tregs), and two recent animal studies suggested that CTLA-4 blockade may function by depleting Treg in vivo [44, 45].

CTLA-4 was the first of the immune checkpoints to be successfully targeted in cancer. Ipilimumab (Yervoy, Bristol-Myers Squibb) is a fully human IgG1 monoclonal antibody-blocking CTLA-4, which received FDA approval in 2011 for improving overall survival in patients with metastatic melanoma [46, 47]. Patients with RCC were included in early trials with CTLA-4-blocking agents [48–50]. In a small phase 2 study of patients with advanced RCC, ipilimumab pro-

Table 16.1 Binding affinity of B7/CD28 and the PD-1/PD-L1 family members and ligands or anti-PD-1-blocking antibodies (taken from the literature, not performed in parallel) demonstrate significant differences in the binding affinity of different checkpoint inhibitors to their target

	Kd	References
PD-1:pembrolizumab	0.028 nM	(Hamid [89] #114) * while Kd 28 pM, 50 % effective binding concentration of pembrolizumab was 0.1–0.3 nM
PD-1:nivolumab	2.6 nM	(Brahmer [81] #48) scatchard plot analysis
PD-1:pidilizumab	20 nM	(Atkins [82] #390)
PD-1:PD-L2	89–106 nM	(Youngnak [130] #433) scatchard plot analysis
PD-1:PD-L1	270–526 nM	(Youngnak [130] #433) scatchard plot analysis
	590–770 nM	(Butte [131] #8) scatchard plot analysis
B7-1:CTLA-4	400 nM	(van der Merwe [132] #432) scatchard plot analysis
B7-1:PD-L1	1,540–1,990 nM	(Butte [131] #8) scatchard plot analysis
B7-1:CD28	4,300 nM	(van der Merwe [132] #432) scatchard plot analysis

*While Kd 28 pM, 50% effective binding concentration of pembrolizumab was 0.1-0.3 nM

duced a 13 % response rate but with a relatively high rate (33 %) of grade 3 and 4 immune-related toxicities, such as enteritis and hypophysitis [51]. Like HD-IL-2, immune-related adverse effects have been reported to be associated with increased rates of antitumor response: ORR in patients with enterocolitis were 36 % for metastatic melanoma and 35 % for RCC, compared with 11 % and 2 %, respectively, in those without enterocolitis. [49]. While the search for predictive biomarkers has yet to produce an actionable biomarker for ipilimumab, a number of pharmacodynamic markers have been reported, such as the absolute lymphocyte count and the percentage of ICOS+ lymphocytes [52, 53]. Gene expression profiling of pretreatment tumors suggests that those with a high-baseline expression levels of immune-related genes, such as T cell markers and chemokines, are more likely to respond to ipilimumab [54].

16.5 Programmed Cell Death-1 (PD-1)

Clinically, antibodies blocking the PD-1/PD-L1 interaction have moved to the forefront of development, due to their higher activity and better tolerability than α-CTLA-4. Unlike with the CTLA-4 models, neither PD-1 nor PD-L1 knockout mice succumb to lethal autoimmunity; instead, some additional insult appears to be required for the development of overt autoimmunity [55–59]. Functionally, PD-1 is an activation marker on T lymphocytes. Activation-induced expression generally declines when antigen is cleared; this has been observed in RCC, as nephrectomy (which presumably eliminates tumor antigen) is associated with a decline in PD-1 levels on T cells [60–62]. As originally described in the lymphocytic choriomeningitis virus (LCMV) model of chronic infection, if an immune response does not successfully eliminate the antigen, prolonged antigen stimulation leads to persistent PD-1 expression [60, 61]. This high expression of PD-1 is associated with an "exhausted," dysfunctional T cell phenotype. Viruses, bacteria, and even parasites may exploit this pathway.

Expression of the major ligand for PD-1 (PD-L1) can be induced in most cell types by the T_H1 effector cytokine IFN-γ; thus, expression is generally associated with inflammation. For example, in HSV keratitis, expression of PD-L1 is upregulated on CD11b+ macrophages [63]. *Schistosoma mansoni* infection also results in upregulation of PD-L1 on macrophages, leading to T cell anergy [64, 65]. Homozygous PD-L1 knockout mice exhibit resistance to the parasite *Leishmania mexicana* [66]. As above, PD-L1 is expressed on many cell types outside of the hematopoietic lineage including cells of the epithelial lineage [35]. The generally mild phenotype of PD-L1 knockout mice, as well as its induction in the context of inflammation, suggests that PD-L1 generally serves to dampen a potentially overexuberant immune response and

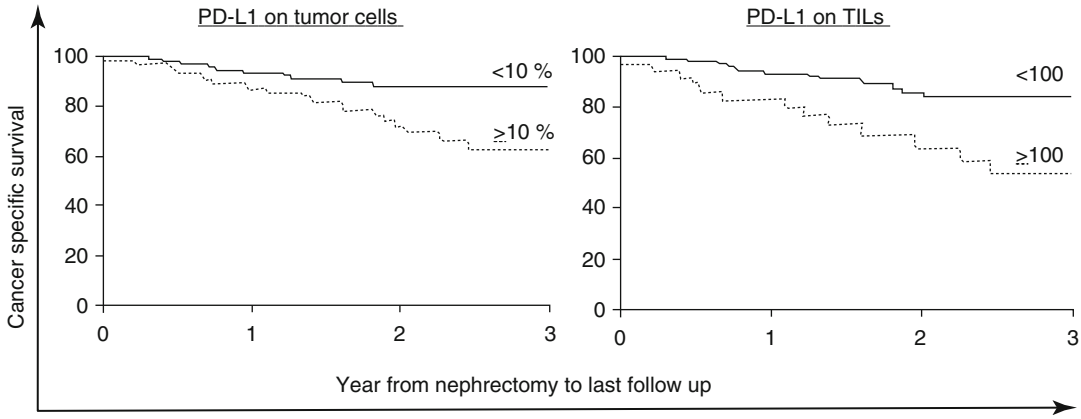

Fig. 16.1 Both PD-L1 on tumor cells and immune infiltrate are associated with death from kidney cancer (Thompson et al. [70]. Printed with permission, Copyright (2004) National Academy of Sciences, USA)

to prevent an anti-pathogen response from leading to autoimmunity [67]. Many tumor types express PD-L1, providing evidence that they have co-opted the PD-1/PD-L1 pathway to protect themselves from immune attack [36].

16.6 Mechanism of Immune Evasion by RCC

RCC is considered an immunogenic tumor as demonstrated by its responsiveness to IL-2 and further supported by anecdotal reports of involution of metastatic disease after removal of the primary tumor [68, 69]. Truly durable responses observed with HD-IL-2 occur in over 50 % of patients who reach a complete remission. However, complete remissions occur in less than 10 % of treated patients. Unfortunately, the majority of patients fail to achieve any benefit from HD-IL-2. Tumors can evade the immune system through both inherent and adaptive resistance to the immune system [70]. Some tumors do not appear to stimulate an active immunologic response in the tumor microenvironment, while other tumors have a lymphocyte-rich milieu. In several cancer types, an increased level of TIL can be a positive prognostic sign, such as colorectal cancer (Jass [71]). In RCC, however, higher levels of PD-L1-positive lymphocytes in tumors and PD-L1 expression on the tumor are generally associated with an increased risk of death from cancer (Fig. 16.1) [72].

As discussed above, one potential mechanism for PD-L1 upregulation in the tumor microenvironment is the secretion of IFN-γ by effector T cells. This upregulation may be considered to be "adaptive," in that some tumors resist T cell attack by adaptively upregulating PD-L1 as a defense mechanism. Support for "adaptive resistance" comes from recent studies in melanoma, where regions of PD-L1 expression are generally in close proximity to areas of active T cell infiltration [73]. The notion of adaptive immune resistance also explains why tumors that express PD-L1 are more likely to respond to PD-1 blockade; such tumors likely have an active, tumor-specific T cell response that is being held in check by the PD-1/PD-L1 axis, so blocking that checkpoint with appropriate antibodies relieves T cell suppression and leads to an effective T cell response, at least in some patients [74].

16.7 Differences Between PD-1 Pathway-Blocking Agents

Blocking either PD-1 or PD-L1 has shown clinical activity in patients with RCC, and there are multiple antibodies under development targeting either PD-1 or PD-L1. While anti-PD-1 antibodies all target the binding site of PD-L1 to PD-1,

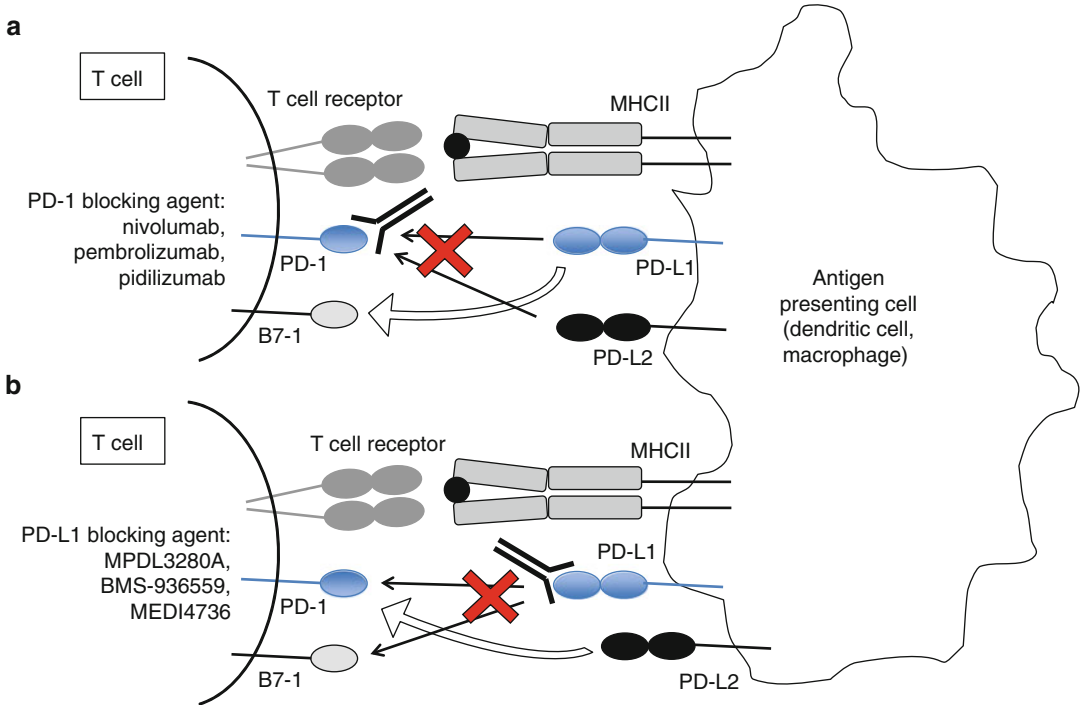

Fig. 16.2 Blocking PD-1 (**a**) or PD-L1 (**b**) may have different clinical effects, since the receptor PD-1 and the ligand PD-L1 have alternative ligands and receptors (*open arrows*), which are not blocked by the therapeutics listed

there may be distinct differences in their clinical benefit. Interfering with the binding site on the receptor PD-1 blocks the interaction between PD-1 and either of its ligands PD-L1 or PD-L2, but not the PD-L1:B7-1 interaction (Fig. 16.2a). Targeting the binding site on the ligand PD-L1 blocks PD-L1 binding of both PD-1 and B7-1, but not the PD-1/PD-L2 interaction (Fig. 16.2b). The different PD-1 and PD-L1 inhibitors have yet to be directly compared in clinical trials. Some have proposed that anti-PD-L1 blockade may produce fewer adverse effects than anti-PD-1 blockade. The safety of combining PD-1 and PD-L1 blockade is being tested in an ongoing phase I trial (NCT02118337).

Variability in clinical benefit among the blocking antibodies may result from intrinsic differences in their structure and engineering. Most antineoplastic antibodies, such as trastuzumab or rituximab, mediate their effects through antibody-dependent cellular cytotoxicity (ADCC) [75]. However, the primary mechanism by which immune checkpoint-targeted antibodies function is by physically blocking the binding of the receptor and the ligand, not by the destruction or depletion of lymphocytes. Indeed, the expression of PD-1 on activated as well as "exhausted" lymphocytes would make it seem unwise to generate a depleting anti-PD-1 antibody, although to our knowledge a PD-1 antibody optimized for depletion has not yet been evaluated in preclinical studies. So, aside from pidilizumab/CT-011, which is an IgG1 isotype antibody, all other PD-L1 and PD-1-blocking antibodies are engineered antibodies with mutated ADCC-activating sites in the Fc domain, or IgG2 or IgG4 antibodies, which have minimal ADCC/CDC activity (Table 16.2).

Indeed, a better understanding of the biology of immune checkpoint blockade may enhance our knowledge about the mechanisms underlying the clinical effects. For example, the checkpoint inhibitors ipilimumab and tremelimumab both block CTLA-4:B7 through binding CTLA-4 on lymphocytes. Like ipilimumab, tremelimumab elicited promising efficacy in early phase I and II studies [76, 77]. Unfortunately, the phase III trial

Table 16.2 Summary of the function and isotype of PD-1 and PD-L1-blocking antibodies currently in development

	Function	Killer isotype	Non-killer isotype
Anti-CTLA4	Blocks the interaction between CTLA-4 and its receptors B7-1 and B7-2	Ipilimumab (IgG1)	Tremelimumab (IgG2)
Anti-PD-1	Blocks the interaction between PD-1 and its ligands PD-L1 and PD-L2	Pidilizumab (IgG1)	Nivolumab (IgG4)
			Pembrolizumab (IgG4)
Anti-PD-L1	Blocks the interaction between PD-L1 and its receptors PD-1 and CD80		BMS-936559 (IgG4)
			MPDL3280A (mutated IgG1[a])
			MEDI4736 (engineered IgG1)

[a]The MPDL3280A antibody is an engineered IgG1, mutated to completely eliminate killer ADCC and CDC activity

of tremelimumab in melanoma showed no significant difference in response rate or overall survival over standard of care chemotherapy [78]. Clinically, the mechanism(s) underlying the antitumor effect of ipilimumab is not fully understood. As mentioned above, recent preclinical studies indicate that the ADCC-activating (killer) Fc domain is necessary for the antitumor effect of anti-CTLA-4 and that a portion of its antitumor efficacy is driven by depletion of T regulatory cells [44, 45]. While CTLA-4 is only transiently expressed on most T cells, T regulatory cells constitutively express CTLA-4. Higher levels of CTLA-4 expression have been found on Tregs in the tumor compared to effector T cells [79]. Interestingly, tremelimumab is an IgG2 isotype (non-killer) antibody, while ipilimumab is an IgG1 isotype (killer) antibody (Table 16.2).

The available PD-1 and PD-L1 antibodies may also vary in their affinity of the antibody for its target. Since all antibodies target the PD-1/PD-L1 binding region, there is probably relatively little difference between the antigen targets of the antibodies. However, the breadth of difference in antibody affinity of three anti-PD-1 antibodies is illustrated in Table 16.1 [80–82]. Of the PD-1-directed antibodies, pembrolizumab has been reported to have the highest affinity for PD-1 (Kd 20 pM), although it is challenging to compare affinities of different antibodies when the assays used to quantify affinity are not uniform. Nonetheless, a higher affinity agent may be a more effective blocking agent and would also allow an antibody to remain efficacious at lower concentrations observed long after administration or at sites with low antibody penetrance.

16.8 Early Studies: Efficacy and Toxicity

16.8.1 Anti-PD-1 Blockade in RCC

16.8.1.1 Nivolumab

Nivolumab is an IgG4 antibody against PD-1 and has been approved in Japan for the treatment of melanoma. The first in-human trial of an anti-PD-1 antibody as a monotherapy treatment in RCC was a pilot phase I, dose-escalation study (BMS-936558, MDX-1106, ONO-4538; Bristol Meyers Squibb). One RCC patient was included; that patient achieved a partial response during the study period [81], but he then went on to develop a long-term complete response (CR) after receiving only three doses of study drug (C. Drake, personal communication). A larger, dose-escalation phase I/II trial of nivolumab included 34 patients with RCC, who were treated every 2 weeks for up to 96 weeks at doses ranging from 0.1 to 10mg/kg [83]. In that phase Ib study, a maximum tolerated dose was not reached in dosages up to 10 mg/kg. Five percent of patients stopped treatment due to toxicity. The immune-related toxicity seen in this study included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis and was not correlated with dose level. Endocrinopathies were generally effectively managed with replacement doses of appropriate hormones. Severe cases of colitis and transaminitis were reversed with treatment interruption or corticosteroids. Pneumonitis was observed in 3 % of all solid tumor patients (9/296). Three cases of pneumonitis-related death were reported (none in RCC patients). These cases prompted strict vigilance for this life-threatening toxicity as well as prompt administration of corticosteroids for clini-

cally significant immune-related adverse events. This enhanced awareness for this adverse event may explain the lack of additional deaths secondary to pneumonitis in subsequent studies. Other adverse effects included fatigue, rash, pruritus, nausea, and decreased appetite. Ten of the 34 patients with heavily pretreated RCC experienced objective responses to nivolumab [84]. Subsequent reporting revealed an encouraging median PFS of 7.3 months and a median OS at 22 months [85].

Two early phase trials have been reported in RCC. A phase II dose-ranging study enrolled 168 patients with RCC with a clear cell component and prior antiangiogenic therapy (NCT01354431) [86]. The type and pattern of adverse effects was similar to previously reported studies. There were no grade 3–4 pneumonitis or grade 5 events reported. Overall response rates were 20–22 % across three dose cohorts. PFS ranged 2.7–4.2 months. OS was perhaps the most encouraging result at 18–25 months. A concurrent phase 1 biomarker trial enrolled 67 patients with clear cell mRCC, who had received 1–3 prior treatments, as well as 24 treatment-naive patients (NCT01358721) [87]. The overall response rate was 17 %. In the previously treated cohort, patients who received the higher dosing of nivolumab at 10 mg/kg had an objective response rate of 22 %. In the untreated patients who were also treated with nivolumab 10 mg/kg, the response rate was 13 %. While these are very small cohorts, the finding of a lower ORR in the treatment-naive cohort was somewhat surprising. A large, phase III randomized trial has accrued and will provide definitive data as to whether nivolumab increases overall survival compared to everolimus in a treatment refractory setting (NCT01668784). Additional studies are ongoing to evaluate whether there is a role for PD-1 pathway inhibitors in the first-line setting of RCC or whether prior VEGF-directed treatment of RCC increases the response rates to PD-L1 blockade.

16.8.2 Anti-PD-L1 Blockade in RCC

16.8.2.1 BMS-936559

The first published clinical trial of PD-L1 blockade reported the initial safety and efficacy of a fully human IgG4-blocking monoclonal antibody against PD-L1, BMS-936559 (Bristol Meyers Squibb) [88]. In this phase I dose-escalation trial (NCT00729664), a maximum tolerated dose was not found, and the maximum dose administered was 10 mg/kg. Immune-mediated adverse events were observed in 39 % of patients, including rash and hypothyroidism, as well as individual cases of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis. Six percent of patients discontinued treatment due to adverse events. Of note, there were no cases of pneumonitis with this anti-PD-L1 antibody. In the subset of patients with renal cell carcinoma, only 2 of 17 patients had objective responses.

Table 16.3 PD-L1 blockade can benefit patients with both clear and non-clear cell RCC, as demonstrated in the phase 1b trial with MPDL3280A (Cho et al. [91])

	n	ORR (%)	24-week PFS (%)
All tumors	140	21	45
RCC	47	13	53
Clear cell RCC	40	13	57
Non-clear cell RCC	6	17	20[a]

[a]While the responses in the cohort with nccRCC is a sample size of 1, further development in these patients is warranted given the lack of effective treatment options for this cohort of patients

16.8.2.2 MPDL3280A

MPDL3280A (Roche/Genentech) is an engineered IgG1 monoclonal antibody directed against PD-L1. This agent has been found to be well tolerated in doses ranging up to 20 mg/kg [89]. In an expansion arm of the phase I study, 15 % of patients with advanced RCC had objective responses with a 24-week PFS rate of 51 % (95 % CI: 38–63) [90]. While most immune checkpoint trials required RCC with a clear cell component, the expansion cohort of MPDL320A also enrolled patients with non-clear cell RCC (nccRCC). Of the six nccRCC patients, one had a partial response (Table 16.3) [91]. Further investigation is warranted in patients with nccRCC as there are few effective treatment options for this subgroup. The clinical benefit of MPDL3280A has also been reported in multiple tumor types and in particular for urothelial carcinoma (UC), which has led to the FDA breakthrough designation for UC patients with PD-L1-positive tumors [92].

16.8.3 Other PD-1 Pathway-Blocking Therapies in Development in RCC

16.8.3.1 Pembrolizumab

Pembrolizumab (MK-3475, Merck) is a humanized anti-PD-1 IgG4 isotype antibody with a high affinity to PD-1 (Table 16.1). It has not yet been evaluated in patients with RCC but is the first PD-1 pathway-blocking antibody to gain FDA approval for patients with ipilimumab-refractory melanoma. Given the excellent efficacy and general tolerability observed in melanoma [80, 93], there are several ongoing clinical trials in which pembrolizumab is being combined with tyrosine kinase inhibitor (TKI) in RCC patients. These include combination with pazopanib (NCT02014636) as well as with axitinib (NCT02133742).

There are also several additional immune checkpoint-blocking antibodies in earlier stages of clinical development. These include MEDI4736, an engineered IgG1 PD-L1 antibody. Preliminary phase I data on this agent were recently reported [94] and included a single patient with RCC who responded for over 36 weeks. Dose-expansion cohorts of MEDI4736 are being studied in multiple other solid tumor types. Pidilizumab (CT-011, CureTech) is a humanized anti-PD-1 IgG1 isotype antibody, which was first tested in patients with hematologic malignancy and was found to be safe and well tolerated at doses ranging from 0.2 to 6 mg/kg [95]. However, a phase II trial in melanoma reported a relatively low overall response rate (approximately 6 %), which is somewhat inconsistent with other PD-1/PD-L1-blocking agents [82]. An ongoing phase II trial is currently assessing the safety of CT-011 alone or in combination with a dendritic cell/RCC cell fusion vaccine (NCT01441765). AMP-224 (Amplimmune and GlaxoSmithKline [GSK]) employs a different strategy for blockade; this agent is a B7-DC immunoglobulin fusion protein, which acts by binding and blocking PD-1. This agent is currently undergoing phase I investigation (NCT01352884).

16.9 Role of PD-L1 Expression in RCC

In several preclinical models, induced expression of PD-L1 on tumors increased tumorigenesis and invasiveness in vivo [15]. In addition to tumor cells, PD-L1 is expressed on infiltrating lymphocytes, monocytes, and macrophages (Fig. 16.3) [72]. In some types of cancers such as ovarian, lung, and RCC, tumoral PD-L1 expression is associated with worse prognosis [72, 96, 97]. In a retrospective cohort of patients with RCC, PD-L1 expression on both tumor cells and immune infiltrate was associated with a relatively poor prognosis (Fig. 16.1) [72]. Patients whose kidney tumors had $\geq$10 % tumor cell expression had a threefold increased risk of dying of their disease. The majority of the patients in this cohort had early-stage resected disease. In a retrospective analysis of the tumors from patients with metastatic disease on the phase III COMPARZ trial comparing sunitinib and pazopanib, PD-L1 expression $\geq$5 % was associated with worse overall survival [98]. An interesting recent study evaluated the expression of PD-L1 on tumor cells and tumor-infiltrating immune cells. These data showed that expression of PD-L1 on infiltrating immune cells varied greatly between melanoma, NSCLC, and RCC and that PD-L1 expression on tumor cells (rather than infiltrating immune cells) had the strongest association with response to nivolumab [99]. These data seem to be contradictory to those reported for the anti-PD-L1 antibody MPDL3280A, where PD-L1 expression on immune cells within the tumor appears to associate most strongly with response [89, 100–101]; however, the two assays employ different detection antibodies and likely different staining protocols, which might explain this discrepancy.

In the phase II study of nivolumab in RCC, higher PD-L1 expression on tumors was associated with an increased likelihood of response, with response rates of 31 % in PD-L1-positive and 18 % in PD-L1-negative tumors (Table 16.4) [102]. It is important to note that the predictive value of PD-L1 expression with nivolumab treat-

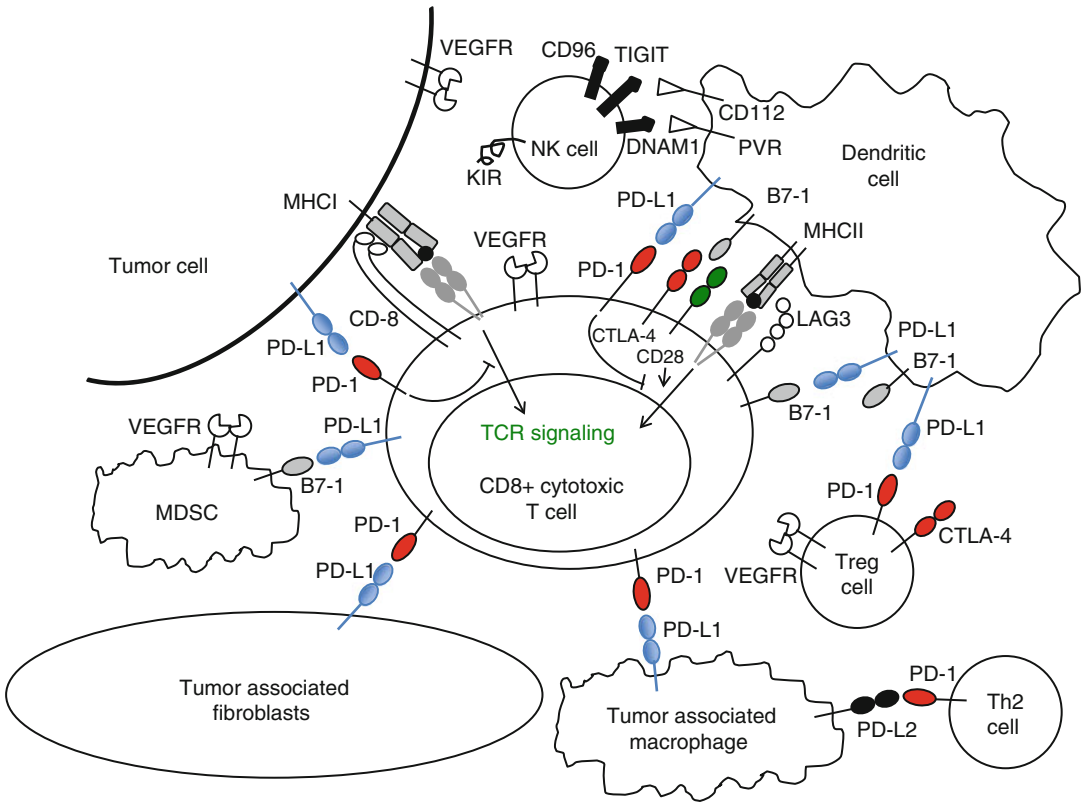

Fig. 16.3 PD-L1 is expressed on many different cells within the immune system. Checkpoint inhibitors may affect other immune cells, in addition to CD8+ effector T lymphocytes in the tumor microenvironment

ment may depend on the antibody clone employed. For example, using an automated assay with the 28-8 antibody clone, the response rate in PD-L1-positive tumors was much lower across tumor types [72, 102–104] than reported in the original study with clone 5H1 [81]. With MPDL3280A, 20 % of RCC patients with PD-L1-positive tumors responded to therapy as compared to 10 % for PD-L1-negative tumors (Table 16.4) [90].

In general, higher PD-L1 expression on tumor cell and immune-infiltrating cells appears to be associated with a higher likelihood of response. However, the use of PD-L1 expression as an upfront selection biomarker is suboptimal, as a significant percentage of "negative" tumors may respond (reviewed in [105]). Furthermore, there are many patients that are "positive" who do not respond. One way in which PD-L1 expression may be useful as a biomarker is for guiding the sequence of therapy or whether monotherapy or combination therapy may be more appropriate—but those questions clearly require prospective investigation. PD-L1-based patient selection is also limited by the lack of a standardized definition of PD-L1 positivity or assay. Other entities (Genentech/Roche, Merck, and MedImmune) who are developing blocking antibodies against either PD-1 or PD-L1 also have developed distinct companion assays for PD-L1 expression each with different anti-PD-L1 antibodies. These assays have yet to be directly compared. Thus, it is impossible to determine whether the differences in correlations found between tumors and treatments reported are a function of the nature of the patient samples analyzed, the biologic differ-

Table 16.4 Summary of clinical outcomes and PD-L1 expression in the development of PD-1 pathway blockade in RCC

Agent(s) (year reported)	Tumor type	*n*	Anti-PD-L1 clone	RR (%) (95 % CI) [n/N]	Pattern defined as positive	RR (%) PD-L1 positive (95 % CI) [n/N]	RR (%) PD-L1 negative (95 % CI) [n/N]	mPFS PD-L1 positive (95 % CI)	mPFS PD-L1 negative (95 % CI)	mOS (m) PD-L1 positive (95 % CI)	mOS (m) PD-L1 negative (95 % CI)
Nivolumab[1] 2012	Solid tumors[a]	42	5H1	40 %[a]	Tumor cell >5 %	36 %	0 %	nr	nr	nr	nr
MPDL3280A[2] 2014	Kidney cancer	62	PDL1dx[b]	14 %	Immune infiltrate >1 %	20 % (9–37)	10 % (2–30)	24 weeks (5–98+)	20 weeks (5–94+)	nr	nr
Nivolumab[3] 2014	Kidney cancer	107	28–8	20–22 %[c]	Tumor cell >5 %	31 % (15.3–50.8)	18 % (10.2–28.3)	4.9 m (1.4–7.8)	2.9 m (2.1–4.2)	NA (13.4 m-NA)	18.2 m (10.2–28.3)
Nivolumab/ Ipilimumab[4] 2014	Kidney cancer	36	28–8	43 % [9] – 48 %[11][d]	Tumor cell >5 % --- >1 %	25 % [1/4] --- 50 % [8/16]	56.3 % [8/16] --- 55 % [11/20]	nr	nr	nr	nr
Nivolumab/ VEGF-TKI[5] 2014	Kidney cancer	33	28–8	52 %[17][e]	Tumor cell >5 % --- >1 %	20 % [1/5] --- 40 % [6/15]	58.3 % [14/24] --- 64.3 % [9/14]	nr	nr	nr	nr

NA not achieved, *nr* not reported

[a]In phase I, of the five patients with RCC, all received 10 mg/kg, and four were PD-L1 positive, of which one had a PR and one had a CR

[b]Genentech/Roche proprietary antibody, positive = IHC 1,2,3, which is equivalent to >1 % positive immune-infiltrating cells

[c]Dose-ranging cohorts: 0.3 mg/kg, 2 mg/kg, 10 mg/kg; confidence interval not reported

[d]Dose-ranging cohorts: Nivo 3 mg/kg+ Ipi 1 mg/kg – 9 pt (43 %, 21.8–66.0); N1+I3 – 11 pt (48 %, 26.8–69.4)

[e]VEGF-TKI cohorts, sunitinib +Nivo −17 pt (52 %, 33.5–69.2); pazopanib arm not shown, 9 pt (45 %, 23.1–68.5); and no significant improvement in ORR in pt with PD-L1-positive arm with pazopanib combo. [1]Topalian et al. *NEJM* [83], [2]McDermott et al., *ESMO* [90], [3]Motzer et al., *ESMO* [102], [4]Hammers et al., *ASCO* [118], [5]Amin et al., *ASCO* [121]

ences of anti-PD-1 versus anti-PD-L1 therapy, or the assay (antibody specificity) itself.

In addition to discrepancies in the assays used, there are many other factors that may complicate the use of tumoral PD-L1 expression as a potential predictive biomarker. Tumor heterogeneity is a significant issue in many tumors, and thus, sampling bias may affect the results [106]. Further, PD-L1 is a dynamic marker that can be upregulated locally in response to cytokines induced by inflammation (adaptive resistance) [35] and in response to the selection pressures of treatment. Thus, the expression of PD-L1 within tumors and its microenvironment may change over time. Therefore the primary tumor expression may not reflect that of the metastases or even the primary tumor's level at a later time [107]. As an example of treatment-induced changes, three similar neoadjuvant phase II trials studied the effects of the VEGF-TKIs sunitinib and pazopanib on the primary tumor and found a reduction in vessel density, PD-L1 expression, and FOXP3 expression, but increases in Fuhrman grade and Ki-67 levels [105]. In addition, PD-L1 can be induced by some oncogenic mutations such as in PTEN (rare in RCC) or directly by gene amplification [109, 107]. Once again, despite these factors, across multiple tumor types using different therapies and varied assays for PD-L1 expression, there is a clear trend for PD-L1-positive tumors to be more likely to respond to blocking antibodies than PD-L1-negative tumors [72, 89, 93, 94, 100–101, 103, 104, 111–113]. It is interesting that preliminary data suggest that PD-L1 expression on RCC tumor cells is also associated with increased clinical benefit from HD-IL-2 [114]. Thus, PD-L1 status could also play a role in building a better predictive model for optimal HD-IL-2 candidates.

16.10 Increasing Response Rates with Combination Therapies

16.10.1 Nivolumab + Ipilimumab

Several trials are underway in which PD-1 pathway blockade is combined with either novel or FDA-approved agents in an effort to build on the clinical efficacy of monotherapy (Table 16.4). Combining PD-1 inhibition with CTLA-4 blockade is one logical approach given preclinical melanoma models showing enhanced efficacy for concurrent combination treatment [115, 116]. The first phase I study combining nivolumab and ipilimumab was performed in advanced melanoma—where encouraging efficacy was observed with an impressive objective response rate of 53 % albeit at the expense of increased toxicity [117].

The combination of nivolumab and ipilimumab is being tested in RCC. Forty-four patients with clear cell RCC were enrolled in two expansion cohorts of the phase 1 trial (NCT01472081). In these cohorts, nivolumab and ipilimumab were administered concurrently for four doses every 3 weeks, followed by maintenance dosing of nivolumab [118]. Two dose regimens were evaluated, one with ipilimumab at 3 mg/kg and nivolumab at 1 mg/kg and a second with ipilimumab at 1 mg/kg and nivolumab at 3 mg/kg. As was observed in the melanoma study, greater toxicity was observed with the higher-dose ipilimumab arm with 61 % of patients (14/23) experiencing a grade 3–4 toxicity as compared to 29 % (6/21) in the ipilimumab 1 mg/kg plus nivolumab 3 mg/kg arm. A higher rate of effects (AEs) immune-related adverse events was also observed in the higher-dose ipilimumab arm including 17 % (4/23) gastrointestinal and 26 % (6/23) hepatic events relative to 5 % (1/21) and 0 % in the lower ipilimumab dose cohort. Treatment discontinuations due to therapy-associated toxicity were 26 % (6/23) and 9.5 % (2/21), respectively. No grade 5 or high-grade pulmonary events, such as pneumonitis, were reported. Across the treatment groups, 43–48 % of patients experienced confirmed objective responses. Despite differences in toxicity, both arms showed essentially equivalent 24-week PFS milestones of 65 and 64 %. Interestingly, PD-L1 status was not predictive of activity for combination therapy; but only 4 of 36 evaluable samples were PD-L1 positive at a 5 % tumor membrane threshold and only one of these 4 patients responded, whereas 18 of 32 patients (56 %) with PD-L1-negative tumors (<5 % membrane staining) had objective responses to combination therapy. Additional exploration of a lower (1 %) cutoff for

PD-L1 positivity showed that response rates were similar in patients with PD-L1-positive (50 %, 8/16) and PD-L1-negative tumors (55 %, 11/20). Based on these intriguing results, a phase 3 study is planned which will test the efficacy of the combination of nivolumab and ipilimumab compared to standard of care sunitinib in treatment-naive patients (NCT02231749).

Given the rapid development in the field, other combinations with immune-modulatory agents are being explored. The impressive responses witnessed with the combination of nivolumab and ipilimumab have prompted the investigation of combinations with multiple other immune modulators, both novel and FDA approved, such as anti-LAG3, 4-1BB, or KIR and IL-21, peginterferon, or lenalidomide. Toxicity has proven to be a concern with combined immune therapy, making careful phase I trials essential for evaluating the safety of these regimens.

16.10.2 PD-1 Plus Antiangiogenic Agents in RCC

VEGF-TKIs are the most frequently used first-line therapy for metastatic RCC. An inverse association has been reported between PD-L1 expression and genes of the VEGF pathway in primary nephrectomy specimens [119]. However, the development of resistance to antiangiogenic therapies is nearly universal. Upon developing resistance to VEGF-TKIs, many tumors produce very high levels of VEGF [120]. Given the high levels of VEGF in most RCC and as well as the immune-modulatory effects of VEGF, combinations with VEGF-TKIs (and nivolumab) or the monoclonal anti-VEGF antibody bevacizumab (and MPDL3280A) are currently underway and have been reasonably well tolerated in early phase trials [121, 122]. In addition to these ongoing studies, a phase 1 study evaluated the combination of nivolumab with either pazopanib or sunitinib (NCT01472081) [121]. Four of the 20 patients in the pazopanib arm experienced DLTs in the form of liver and renal toxicity, and thus, enrollment to that arm was halted. The sunitinib combination was better tolerated, and after an initial 14 pretreated patients were enrolled, the dose expansion cohort was expanded to allow 19 treatment-naive patients. In all, no grade 5 treatment-related AEs were observed; however, 81.8 % (27/33) and 70 % (14/20) of patients suffered grade 3–4 toxicities, most of which were consistent with TKI therapy, including hypertension, transaminitis, hyponatremia, leukocytosis, diarrhea, and fatigue. Presumed nivolumab-related AEs included endocrine, gastrointestinal, hepatic, pulmonary (2 pneumonitis events, one grade 3–4), skin, and renal AEs. Transaminitis was the most frequent grade 3–4 toxicity. The liver and kidney insults occurred more frequently than expected with either agent alone. Confirmed ORR were 52 % (CI 33.5–69.2) and 45 % (CI 23.1–68.5) in the sunitinib and pazopanib containing arms, respectively. These ORR are higher than expected with each agent individually, although formal testing for synergy is not possible in a single-armed trial. If PD-1 blockade proves effective for RCC, the optimal sequence or combinations of therapies will need to be determined, because it is not clear if the clinical benefit is additive or synergistic. This is particularly relevant when keeping in mind the melanoma experience where patients, who were treatment naive or who has less tumor burden, had better response rates to PD-1 blockade [104].

Combination therapy with the FDA-approved anti-VEGFR antibody bevacizumab is also being investigated. The hypoxic microenvironment can significantly affect immune function [123], and normalization of the vasculature with more directed VEGF therapy, such as bevacizumab, may be effective [124]. A series of studies has found that soluble VEGF levels are associated with shorter PFS and overall survival [1, 125, 126]. In addition to endothelial cells, VEGF receptors are expressed on some immune cells, including cytotoxic and regulatory T lymphocytes and myeloid-derived suppressor cells (MDSC) (Fig. 16.3). The tumor microenvironment can induce immunosuppressive myeloid cells [127]. In addition, MDSC's proangiogenic and immunosuppressive effects have been described as a mechanism of resistance to the VEGF-TKI sunitinib in RCC [128]. Recently, the preliminary safety profile for MPDL3280A in combination with bevacizumab in RCC was reported in a very small subset of RCC patients,

and the combination was found to be generally tolerable with no unexpected toxicities [122]. Of the ten patients evaluable, four had a partial response, five had stable disease, and one patient had primary refractory disease. This combination thus warrants further investigation in larger numbers of RCC patients [122]. It is unclear from the small numbers tested in these phase I studies of non-comparative nivolumab and MPDL3280A or VEGF-TKIs combination whether prior antiangiogenic therapy increases PD-L1 tumor expression or the tumors' likelihood of responding to therapy. It will also be interesting to determine whether efficacy and durability will be either additive or synergistic relative to single-agent therapy.

16.11 Future Clinical Considerations

PD-1 pathway blockade in RCC is a burgeoning field with many open questions as to the optimal patient selection, sequencing, and combination therapies for maximizing patient benefit. As current trials mature, new trials open, and more effective therapies are developed; the reality of significant improvement in durable responses and overall survival for the majority of patients with metastatic RCC appears potentially attainable. The best metric for clinical outcome with these agents has yet to be determined and may depend on the goal of therapy, i.e., prolonged survival or complete response. Given the delayed responses or "flare" immune phenomena that can be observed with these agents, there is a subset of patients that benefit from immune checkpoint inhibition that may not be captured by traditional response criteria, and their true clinical benefit may be underestimated [129]. Collectively, across several tumor types, the remarkable antitumor effect seen with the immune checkpoint inhibitors in tumors refractory to standard therapies is a landmark in oncology. Optimal trial design for immune therapies may depend on developing new standards for measuring tumor response and the development of a predictive biomarker that will better select which patients will benefit from these therapies alone or in combination. The development of the VEGF-TKIs and the mTor inhibitors transformed the treatment of RCC in the last decade by prolonging survival for many patients. The early efficacy observed with the immune checkpoint strategies has energized the field, and one can envision the next decade as an immunotherapy revolution with combinations of these agents and identification of predictive biomarkers bringing us closer to the cure in RCC.

Clinical Vignette

W.J. presented at age 60 with hematuria and was subsequently found to have a 6 cm renal mass and 1 cm lung nodule. He underwent radical nephrectomy, renal vein thrombectomy, retroperitoneal lymph node dissection, and a concurrent left lower lobe wedge resection. Pathology confirmed a diagnosis of fuhrman grade 3, pT3bN0M1 clear cell RCC. As resection was felt to be complete, he was followed with routine imaging studies. CT scans revealed no evidence of recurrence until approximately 2 years post-resection, when disease in a hilar lymph node became apparent. At the time of recurrence, he had an excellent performance status and elected to undergo treatment with high-dose interleukin-2. Unfortunately, imaging studies obtained after his first treatment cycle revealed progression.

At the time of progression, W.J. was consented and enrolled on a phase I trial of an anti-PD-1 antibody (nivolumab, MDX-1106). He received the study drug at a dose of 1mg/kg every 2 weeks, in an 8-week cycle. Initial on-study imaging studies (day 56) showed multiple new liver lesions (arrows) and an increase in his hilar lymphadenopathy (open arrow) (Fig. 16.4c, d). Since the patient remained asymptomatic, he was continued on treatment, per protocol. Imaging after the second cycle of treatment (at 16 weeks) showed improvement in the hilar lymphadenopathy and resolution of his liver lesions. He continued to have improvement

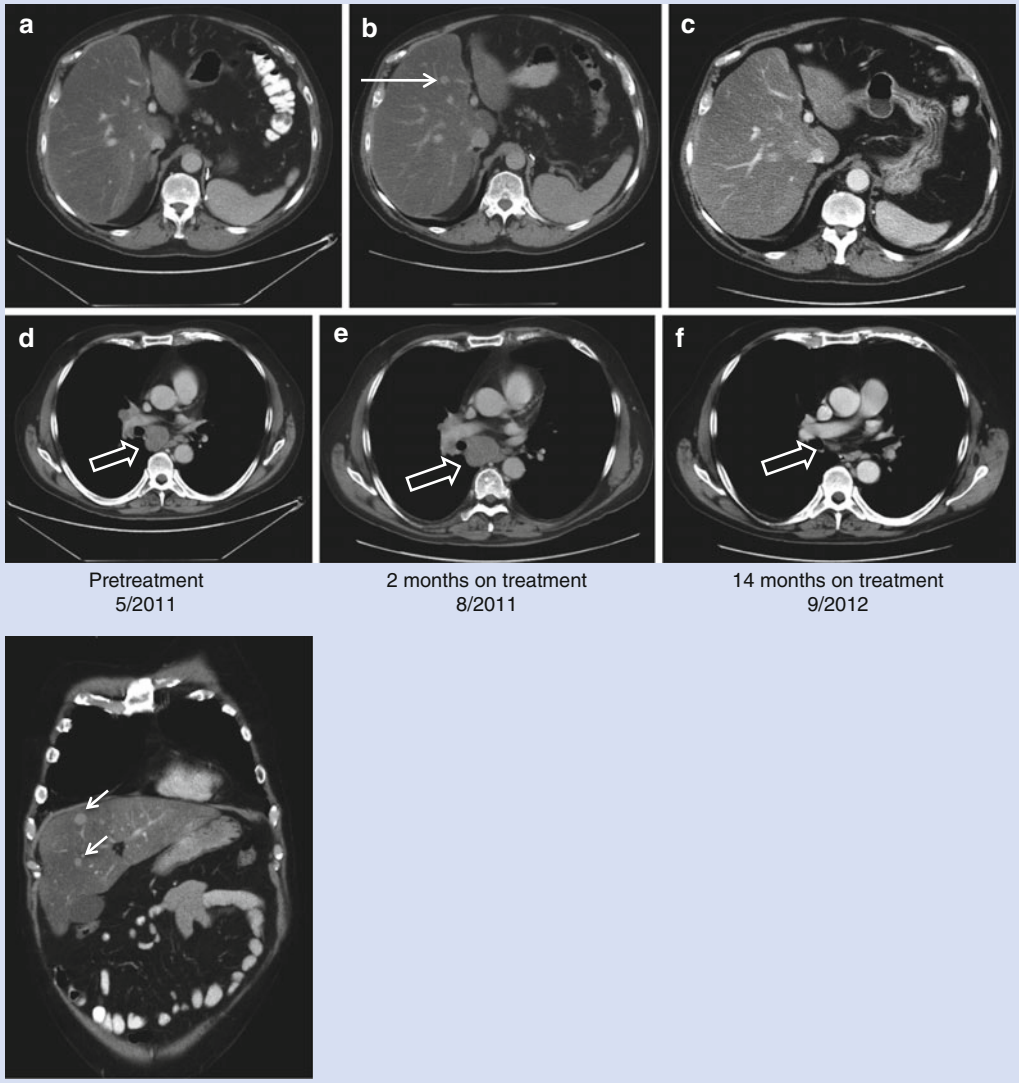

Fig. 16.4 The caption would be "2 months on treatment 8/2011"

in his disease for over a year (cycle 9; Fig. 16.4e, f), after which his disease slowly progressed. He was continued on therapy until he developed grade 2 treatment-related colitis after 17 months on trial. After biopsy confirmation of active colitis, he was started on prednisone at 60 mg orally daily, and his diarrhea slowly improved over 2 weeks. He continued a slow taper for 1 month without recurrence of his GI symptoms.

This case illustrates several unique features of immune checkpoint inhibitor therapy in a patient with RCC, including a response after initial progression and the frequent durability of responses. In addition to efficacy, it also highlights the real possibility of immune-related adverse events and how early recognition of immune-related adverse events and prompt treatment generally render these events reversible.

Disclosures Kathleen Mahoney reports no conflict of interest with this work. Lauren Genentech to my list of advisory Boards has participated in advisory boards for Bristol-Myers Squibb, Pfizer, Aveo, Medivation/Astellas, and Dendreon. She received research funding or support from Medivation/Astellas and Dendreon. Virginia Seery reports no conflict of interest with this work. Charles G. Drake reports receiving a commercial research grant from Bristol-Myers Squib and Janssen; has ownership interest in Compugen, NexImmune, and Potenza; and is a consultant/advisory board member for Bristol-Myers Squibb, Compugen, Dendreon, Roche/Genentech, Novartis, and Merck. Patents licensed: Potenza.

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Angiogenesis Inhibitor Therapy in Renal Cell Cancer

17

Jonathan Mathias and Brian Rini

Contents

Key Points

- Angiogenesis is a key pathway in renal cell carcinoma (RCC), and disrupting this pathway by targeting vascular endothelial growth factor (VEGF) and the VEGF receptor is a viable therapeutic strategy.
- Tyrosine kinase inhibitors (TKIs) such as pazopanib or sunitinib are small molecules that bind to the VEGF receptor (VEGF-R), while bevacizumab and aflibercept bind the VEGF ligand.
- Although no clinical trial has unequivocally demonstrated an overall survival benefit for these agents, the overall survival of metastatic RCC patients has improved dramatically in the targeted therapy era.
- Sunitinib and pazopanib are VEGF-R TKIs that are approved and commercially available for first-line treatment of mRCC, while axitinib and sorafenib are approved in the refractory setting.
- Bevacizumab in combination with interferon is approved for the frontline treatment of mRCC.
- The choice of agent for the first-line treatment of mRCC should be made on an individual basis considering side effect profile, administration route, and patient preference.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_17

17.1 Introduction

Our modern understanding of the molecular biology of renal cell carcinoma (RCC) has established the role of the vascular endothelial growth factor (VEGF) pathway as a relevant therapeutic target. As a result, the management of renal cell carcinoma has undergone a transformation in recent years. Metastatic RCC has witnessed the greatest change, with the addition of VEGF-targeting agents to the clinician's tool kit. This chapter provides a review of the role of VEGF in RCC as well as the major clinical trials that have resulted in changes in standard of care in this disease.

Historical Note

In 1945 a paper by Algire et al. suggested that tumor cells could elicit continuous growth of the new capillary endothelium in vivo [1]. In a series of discoveries, by 1968, scientist had shown that in vitro, tumor tissue cannot grow beyond a certain size (3–4 mm) without neovascularization – a process which did not require direct tumor cell contact as demonstrated by experiments using a biological filter [2–6]. In a carefully designed experiment using Walker 256 ascites tumor in a rat model, Folkman was able to demonstrate the existence of a mitogenic factor that promotes angiogenesis [7]:

> Human and animal solid tumors elaborate a factor which is mitogenic to capillary endothelial cells. This factor has been called tumor angiogenesis factor [TAF]. The important components of TAF are RNA and protein. It is suggested that blockade of this factor (inhibition of angiogenesis) might arrest solid tumors at a tiny diameter of a few millimeters.

He further developed his insight into the potential role of angiogenesis in the treatment of solid tumors and, by publication of his seminal paper in 1971, ushered in a new era of research in cancer treatment [8]. Today, Folkman (1933–2008) is known as the father of the angiogenesis cancer theory. He lived to see the fruits of his theory in the form of pharmacologic agents that constitute some of the most important tools available to the oncologist today.

17.2 Angiogenesis Targeting VEGF in RCC

The pathogenesis of RCC was elucidated by the discovery of the von Hippel–Lindau (*VHL*) gene from the study of VHL syndrome families [9]. Angiogenesis is an essential component of tumor growth and metastasis and central to this process is the VEGF pathway. VEGF is regulated by several growth factor pathways, including hypoxia. Several oncogenes have been demonstrated to upregulate basal levels of VEGF. The main pathway regulating gene induction in response to hypoxia is under the control of the transcription factors HIF-1α and HIF-2α [10–12]. HIF-1α and HIF-2α are, in turn, regulated by ubiquitin-mediated proteolysis and are targeted for destruction by the VHL protein in normoxia and stabilized under hypoxia [13–16]. In sporadic RCC, *VHL* gene allele inactivation, through mutation or promoter methylation, has been shown in 84–98 % of cases [17]. Mutations in the *VHL* gene, as in sporadic renal cancer and VHL syndrome, result in expression of HIF-1α and HIF-2α in normoxia and a permanent transcriptional induction of hypoxia-responsive genes, most notably VEGF (Fig. 17.1) [15].

17.3 Inhibition of VEGF in Renal Carcinoma

The above data provide evidence for *VHL* gene inactivation in the majority of clear cell RCC tumors, which leads to overexpression of VEGF and other factors as a driving force in renal tumor angiogenesis. In fact, RCC develops highly vascular features in both the primary and metastatic sites of disease. Thus, with the development of effective agents targeting the angiogenesis signaling pathway, inhibition of VEGF has been aggressively pursued as a therapeutic target in RCC.

17.3.1 Sorafenib

Sorafenib (Nexavar®, Onyx Pharmaceuticals, Inc., and Bayer Pharmaceuticals Corp.) is an

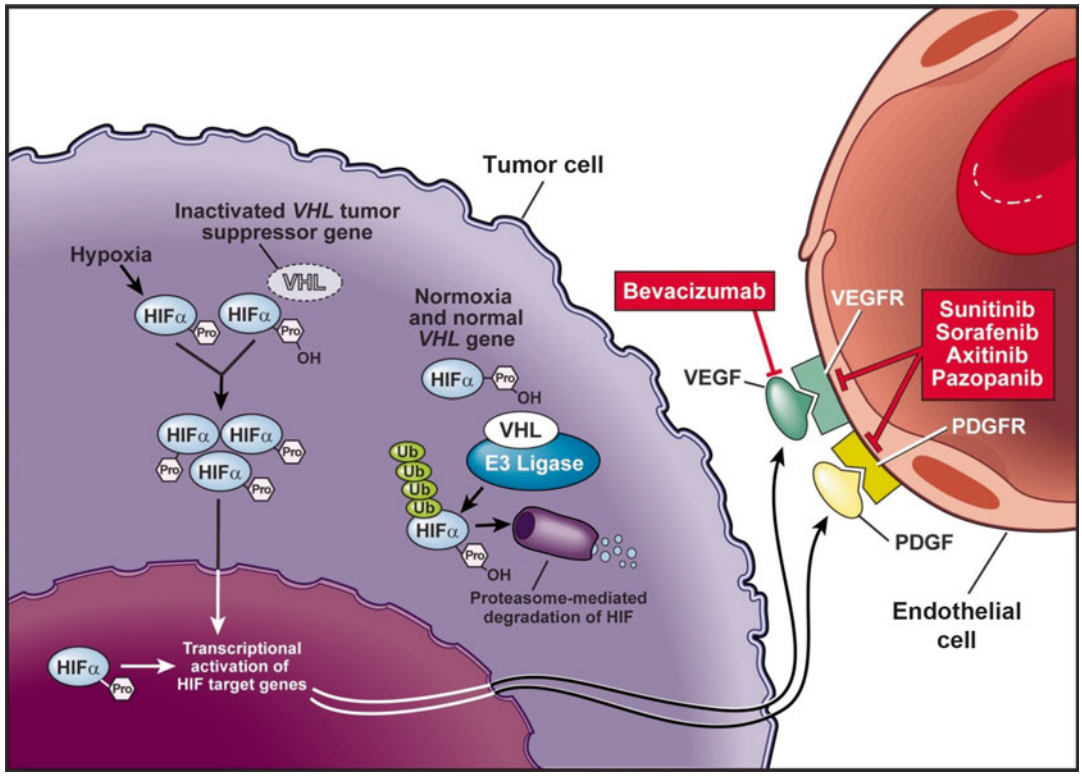

Fig. 17.1 RCC biology which leads to VEGF upregulation. Therapeutics are listed in the *red boxes* that target major elements of this pathway

inhibitor of VEGF receptor 2, FLT3, c-Kit, platelet-derived growth factor receptor (PDGF-R), fibroblast growth factor receptor-1 (FGF-R-1), CRAF, and both mutant and wild-type BRAF [18]. It received FDA approval on December 20, 2005. This approval was granted based on the results of a phase III study in 905 patients with advanced renal cell carcinoma who had received one prior systemic treatment with endpoints of overall survival (OS), progression-free survival (PFS; primary endpoint), and response rate. Patients with ECOG performance status (PS) of 0 or 1, and favorable or intermediate Memorial Sloan Kettering Cancer Center (MSKCC) prognostic risk category were eligible for enrollment. Sorafenib improved the median progression-free survival (PFS) to 5.5 months vs. 2.8 months in the placebo group (HR = 0.44; 95 % CI, 0.35–0.55; $p < 0.001$). The observed benefit in progression-free survival (PFS) was independent of age, MSKCC score, previous use of cytokine therapy, presence of lung or liver metastases, as well as the time since diagnosis (<1.5 or ≥1.5 years). The median overall survival in the sorafenib group in this trial was 19.3 months vs. 15.9 months in the placebo group (HR = 0.77; 95 % CI, 0.63–0.95; $p = 0.02$); however, this result did not reach the pre-specified O'Brien–Fleming boundaries for statistical significance [19]. However, after censoring the placebo patients who crossed over to the [sorafenib] arm, there was a suggestion of improved OS with sorafenib (17.8 versus 14.3 months; $p = 0.029$).

Among the 451 patients assigned to sorafenib (of the total of 903 patients in the trial), 18 patients (4 %) discontinued therapy for adverse events. The most common adverse events were diarrhea (43 %), rash (40 %), fatigue (37 %), hand–foot syndrome (30 %), nausea (23 %), alopecia (27 %), pruritus (19 %), and hypertension (17 %). Anemia was reported in 8 % of the patients receiving sorafenib [19].

A clinical trial of sorafenib 400 mg twice daily vs. INF-α in first-line treatment of mRCC was conducted to further explore the activity of sorafenib in the frontline setting. Sorafenib did not show any PFS benefit (5.7 months in sorafenib vs. 5.6 months in INF-α; HR = 1.14; 95 % CI, 0.79–1.64; $p = 0.504$). However, patients on sorafenib had better quality of life indices. Additionally, on dose escalation to 600 mg twice daily after progression of disease (PD) on the lower dose (400 mg twice daily), there was an additional PFS of 3.6 months. Patients who had PD on INF-α crossed over to sorafenib 400 mg twice daily and had an additional PFS of 5.3 months [20]. These data have tempered the enthusiasm for sorafenib in the frontline setting, although it is still a viable option owing to the overall good tolerability.

With other drugs having shown superior efficacy in first-line and second-line setting, sorafenib's use has been relegated to individuals who have had disease progression after being on newer VEGF-R-targeted therapy. More recently, sorafenib was compared to temsirolimus, an MTOR inhibitor, in a second-line setting for patients who had disease progression on sunitinib. Sorafenib demonstrated superior OS (stratified HR, 1.31; 95 % CI, 1.05–1.63; two-sided $p = 0.01$) and median OS in sorafenib and temsirolimus arms was 16.6 months and 12.3 months. The safety profiles of these drugs were similar to those seen in previous studies [21]. While the precise reason for the OS advantage to sorafenib is unclear, these data have largely been interpreted to support the concept of sequential VEGF-targeting therapy in metastatic RCC.

17.3.2 Sunitinib

Sunitinib (Sutent®, Pfizer, Inc.) is a potent inhibitor of VEGF-R types 1–3, FLT3, c-Kit, PDGF-R-α, and PDGF-R-β [22]. It received FDA approval on January 26, 2006. Two initial phase II trials of sunitinib (50 mg/day for 4 weeks followed by 2 weeks rest) in a total of 169 metastatic RCC patients who had failed prior cytokine-based therapy demonstrated an investigator-assessed objective response rate of 45 %, a median duration of response of 11.9 months, and a median PFS of 8.4 months [23, 24]. This was later converted to regular approval based on an improvement in progression-free survival (PFS) in a randomized phase III first-line therapy setting [25]. Previously untreated mRCC patients ($n = 750$) with clear cell histology were randomized 1:1 to receive sunitinib 50 mg once daily, in 6-week cycles consisting of 4 weeks of treatment followed by 2 weeks without treatment or IFN-α as a subcutaneous injection three times per week on nonconsecutive days at 3 MU per dose during the first week, 6 MU per dose the second week, and 9 MU per dose thereafter. The primary endpoint was PFS (from historical control of 4.7–6.2 months). Secondary endpoints included objective response rate, overall survival, and safety. Health-related quality of life was also assessed with the use of the Functional Assessment of Cancer Therapy–General (FACT-G) and FACT–Kidney Symptom Index (FKSI) questionnaires. Patients were stratified according to baseline levels of LDH, ECOG performance status, and the presence or absence of nephrectomy. The objective response rate by investigator review was 47 % in the sunitinib group versus 12 % in the IFN-α group; $p < 0.001$. Similarly, the median PFS by third-party independent review was 11 months versus 5 months in favor of sunitinib-treated patients corresponding to an HR of 0.42 (95 % CI, 0.32–0.54; $p < 0.001$). Sunitinib-treated patients had a greater median OS when compared with the IFN-α group (26.4 months; 95 % CI, 23.0–32.9 months, versus 21.8 months; 95 % CI, 17.9–26.9 months, respectively; HR, 0.821; 95 % CI, 0.673–1.001; $p = 0.051$) based on the primary analysis of the unstratified log-rank test ($p = 0.013$ using the unstratified Wilcoxon test). By stratified log-rank test, the HR was 0.818 (95 % CI, 0.669–0.999; $p = 0.049$). More than 50 % of patients in both arms of this trial went to receive subsequent treatment with a VEGF-targeted agent including sunitinib, perhaps accounting for the lack of statistical significance observed in the pre-specified OS analysis. The results of this trial have positioned sunitinib as a standard frontline therapy for

mRCC patients. The main limitation of the approved regimen of a 6-week cycle of 50 mg/day for 4 weeks followed by 2 weeks off therapy was toxicity. Seventy patients (19 %) in the sunitinib treatment arm ($N=375$) discontinued treatment for adverse events. Diarrhea, fatigue, and nausea were seen in more than 50 % and hypertension and hand–foot syndrome in approximately 30 % of patients on sunitinib. Laboratory abnormalities included anemia (79 %), neutropenia (77 %), and thrombocytopenia (68 %) [26].

A recent randomized phase II trial examined the standard dosing of sunitinib (Arm A) vs. continuous dosing at 37.5 mg daily (Arm B) [27] in the first-line management of mRCC. The primary endpoint was time to tumor progression (TTP) and the secondary endpoints included objective response rate (ORR), overall survival (OS), and adverse events. Two hundred and ninety-two patients were randomized equally to both arms. Median TTP was 9.9 vs. 7.1 months in Arms A and B, respectively (HR = 0.773; 95 % CI, 0.57–1.04; $p=0.090$). ORR and OS were not statistically significantly different, although numerically favored the 50 mg 4/2 regimen. The most common adverse events were fatigue 65 % vs.71 % in both groups, nausea 63 % vs. 54 %, and diarrhea 59 % vs. 69 % [28]. These data support that 50 mg 4/2 is the preferred dose and schedule and that lower doses do not improve tolerability and may compromise clinical outcome. Alternative schedules of sunitinib such as 2 weeks on followed by 1 week off have been shown to decrease toxicity and better balance the benefits and side effects of this agent with planned prospective trials of alternative schedules [29].

To study the role of sunitinib in the second-line setting for mRCC patients who had failed prior bevacizumab-based therapy, a small ($n=61$) phase II trial was conducted [30]. Tumor burden reduction was observed in 85 % of patients including 14 patients (23 %) who achieved a RECIST-defined PR. The median PFS was 30.4 weeks (95 % CI, 18.3–36.7 weeks) and median OS was 47.1 weeks (95 % CI, 36.9–79.4 weeks). In this study, prior response to bevacizumab did not predict for subsequent response or lack thereof to second-line sunitinib treatment. These data support the empiric current practice of sequential VEGF-targeted monotherapies in metastatic RCC patients.

17.3.3 Pazopanib

Pazopanib (Votrient™, GlaxoSmithKline) is an oral angiogenesis inhibitor with multiple targets including vascular endothelial growth factor receptor (VEGF-R), platelet-derived growth factor receptor (PDGF-R), and c-Kit. It received FDA approval on October 19, 2009, after a phase I clinical trial established the MTD and DLT of pazopanib in refractory solid tumors [31]. A multicenter phase II trial examined the efficacy and safety of pazopanib (800 mg orally daily) in 225 mRCC patients [32]. This study was originally designed as a randomized discontinuation trial. However, after planned interim analysis conducted after the first 60 patients completed 12 weeks of treatment demonstrated a response rate of 38 %. Based on this activity and on recommendation by the independent DSMB, randomization was halted, and all continuing patients in the study were treated on an open-label basis. The ORR observed was 35 % (95 % CI, 28–41 %) by independent review. This was similar regardless of previous treatment or not (37 % versus 34 %, respectively). The estimated median PFS for the entire cohort was 45 weeks (95 % CI, 36–59 weeks). Although the toxicity profile was similar to that seen with other small VEGF-R inhibitors, grade 3 AST and ALT elevation were noted in 6 % and 4 %, respectively, and have emerged as a somewhat unique side effect to this agent.

FDA approval was granted based on a randomized placebo-controlled phase III trial in 435 patients previously untreated or treated with cytokine therapy; most patients were good or intermediate risk group. This clinical trial found that pazopanib compared to placebo significantly prolonged PFS in the overall study population (median PFS 9.2 vs. 4.2 months; HR = 0.46; 95 % CI, 0.34–0.62; $p<0.0001$), in the treatment-naïve subpopulation (median PFS 11.1 vs. 2.8 months; HR = 0.40; 95 % CI, 0.27–0.60; $p<0.0001$), and

in the cytokine-pretreated subpopulation (median PFS 7.4 vs. 4.2 months; HR = 0.54; 95 % CI, 0.35–0.84; $p < 0.001$). The objective response rates in this clinical trial were 30 % in the pazopanib group vs. 3 % in the placebo group with a median duration of responses of 59 weeks [33]. Among the 290 patients assigned to pazopanib (of the total of 435 patients in the trial), 41 patients (14 %) discontinued therapy for adverse events. The most common adverse events were diarrhea (52 %), hypertension (40 %), hair color changes (38 %), nausea (26 %), and fatigue (19 %). Abnormal ALT and AST (53 %), hyperglycemia (41 %), neutropenia (34 %), and thrombocytopenia (32 %) were among the more common laboratory abnormalities reported with use of pazopanib [33].

Pazopanib and sunitinib were compared head to head in a first-line setting in the COMPARZ trial, a non-inferiority trial for patients with metastatic clear cell renal cell carcinoma. One thousand one hundred patients were randomized to receive either pazopanib 800 mg daily or sunitinib 50 mg daily (4-week-on, 2-week-off schedule). Pazopanib was shown to be non-inferior with respect to PFS (HR, 1.05; 95 % CI, 0.90–1.22). Overall survival was found to be similar (HR, 0.91; 95 % CI, 0.76–1.08). Patients treated with sunitinib had higher incidence of fatigue (63 % vs. 55 %), hand–foot syndrome (50 % vs. 29 %), and thrombocytopenia (78 % vs. 41 %). Patients on pazopanib were more likely to have abnormalities in liver function tests (ALT elevations 60 % vs. 43 %). In 11 out of 14 health-related quality of life domains, treatment favored pazopanib ($p < 0.05$) [34].

Pazopanib and sunitinib were also compared in a randomized, controlled, double-blind crossover trial (PISCES) looking at patient preference. Pazopanib was preferred to sunitinib by patients (70 % vs. 22 %) with less fatigue and overall better quality of life being the main factors [35]. These data have led to some increase in the front-line use of pazopanib although sunitinib is still more commonly used. The data show that both agents are effective in this setting with some differences in tolerability that may allow for individualization of therapy.

17.3.4 Bevacizumab

Bevacizumab (Avastin®, Genentech, Inc.) is a monoclonal antibody that binds to and neutralizes circulating VEGF. It received FDA approval on July 31, 2009, in combination with interferon alpha (IFN-α) for the treatment of patients with metastatic renal cell carcinoma. The approval was based on the results from two multicenter phase III clinical trials of previously untreated patients with metastatic renal cell carcinoma. The AVOREN study was an international phase III trial that randomized 649 untreated mRCC patients to receive treatment either with IFN-α (Roferon; Roche, Basel, Switzerland) plus placebo or interferon plus bevacizumab [36]. Patients had predominant (>50 %) clear cell histology and had undergone a previous nephrectomy. Bevacizumab 10 mg/kg or placebo was administered intravenously every 2 weeks with no dose reductions permitted. IFN-α 9 MIU was administered three times per week as a subcutaneous injection. The study was designed to detect an OS improvement from 13 to 17 months with PFS, ORR, and safety as secondary endpoints. Due to the change in standard of care and the availability of other active VEGF inhibitors which precluded reaching the anticipated OS endpoint, the study was amended and unblinded at the time of final PFS analysis. The median PFS observed was 10.2 months in the bevacizumab plus IFN-α group, compared with 5.4 months in the control group (HR, 0.63; 95 % CI, 0.52–0.75; $p = 0.0001$); a significant ORR difference was also observed in favor of the bevacizumab-treated patients (31 % vs. 13 %; $p < 0.0001$). The final median OS was 23.3 months in the bevacizumab arm compared to 21.3 for the IFN-α plus placebo-treated arm (HR, 0.86; 95 % CI, 0.72–1.04; stratified log-rank test $p = 0.1291$).

A second multicenter phase III trial, which was conducted in the United States and Canada through the Cancer and Leukemia Group B (CALGB 90206) [37, 38], was nearly identical in design with the exception that it lacked a placebo infusion and did not require prior nephrectomy. This trial enrolled 732 untreated mRCC patients (369 to bevacizumab plus IFN-α and 363 to

IFN-α alone). The primary endpoint of the study was to detect a 30 % improvement in OS in patients randomly assigned to bevacizumab plus IFN-α compared to IFN-α monotherapy. The median PFS of the study was 8.5 months in patients who received bevacizumab plus interferon versus 5.2 months for patients who received interferon monotherapy ($p<0.0001$). The hazard ratio for progression in patients who received bevacizumab plus interferon after adjusting for stratification factors was 0.71 ($p<0.0001$). Moreover, among patients with measurable disease, the ORR was higher in patients who received bevacizumab plus interferon (25.5 %) than for patients who received IFN-α monotherapy (13.1 %; $p<0.0001$). The median OS in this study was 18.3 months for bevacizumab-treated patients compared to 17.4 months for those receiving IFN-α alone ($p=0.069$). The contribution of interferon to the antitumor effect of this regimen currently is unclear as neither study contained a bevacizumab monotherapy arm, precluding evaluation of the risk/benefit of the addition of cytokines. Similarly, the appropriate dose of IFN-α when given in combination with bevacizumab remains unknown, notwithstanding the fact that a significant percentage of patients receiving the bevacizumab-containing regimen in both phase 3 trials required dose modifications of IFN-α. A recent exploratory analysis of the AVOREN study would suggest that the improvement of PFS observed with the addition of the VEGF antibody to IFN-α appears to be maintained in spite of the need for IFN-α dose reductions (10.2 months with full dose vs. 12.4 months in patients who required a reduced dose of IFN-α) [36]. Given the lack of dose response for interferon, it is possible that lower interferon doses in this combination can reduce toxic effects and preserve efficacy. Such a hypothesis requires prospective testing.

Among the 325 patients assigned to bevacizumab (of the total of 649 patients in the trial), 86 patients (26 %) discontinued therapy for adverse events. The most common adverse events were pyrexia (45 %), anorexia (36 %), fatigue (33 %), bleeding (33 %), asthenia (32 %), hypertension (26 %), flu-like illness (24 %), and diarrhea (20 %). Proteinuria (18 %) and neutropenia (7 %) were among the more common laboratory abnormalities reported with use of bevacizumab. The use of bevacizumab as frontline therapy has been limited by the need for IV infusion and the phase III data which supports the concomitant use of IFN-α.

17.3.5 Axitinib

Axitinib (Inlyta® Pfizer, Inc) is an oral selective inhibitor of vascular endothelial growth factor receptors (VEGF-R) 1, 2, and 3. Data from a multicenter, open-label, phase II study of patients with sorafenib-refractory mRCC who received a starting dose of axitinib 5 mg orally twice daily with a primary endpoint of objective response rate (ORR) provided evidence of activity of axitinib in this disease. Out of 52 patients which enrolled, 2 complete and 21 partial responses were seen with an ORR of 44.2 % (95 % CI, 30.5–58.7). Median response duration was 23.0 months (20.9, not estimable; range 4.2–29.8). Median time to progression was 15.7 months (8.4–23.4, range 0.03–31.5) [39].

In another phase II trial, 62 patients were recruited and the ORR was 22.6 %, and the median duration of response was 17.5 months. The median PFS was 7.4 months (95 % CI, 6.7–11.0 months), while the median OS was 13.6 months (95 % CI, 8.4–18.8 months). Grade 3–4 adverse events included hand–foot syndrome (16.1 %), fatigue (16.1 %), hypertension (16.1 %), dyspnea (14.5 %), diarrhea (14.5 %), dehydration (8.1 %), and hypotension (6.5 %) [40].

Axitinib gained FDA approval in 2012 based on a study where it was compared to sorafenib in a phase III trial with patients who had mRCC refractory to one prior first-line regimen (AXIS trial). Seven hundred twenty-three patients were randomly assigned to receive either axitinib (5 mg bid $n=361$) or sorafenib (400 mg bid $n=362$). Axitinib was associated with a more favorable PFS (6.7 months vs. 4.7 months; HR, 0.665; 95 % CI, 0.544–0.812; one-sided $p<0.0001$). Common side effects were diarrhea,

hypertension, dysphonia, and nausea seen in the axitinib arm and HFS, diarrhea, and alopecia in the sorafenib arm [41].

A separate phase III trial in treatment-naïve or cytokine-refractory metastatic RCC patients compared axitinib to sorafenib in metastatic RCC, and it did not show a significant improvement in PFS (10.1 months vs. 6.5 months; stratified HR, 0.77; 95 % CI, 0.56–1.05). Any-grade adverse events that were more common (≥10 % difference) with axitinib than with sorafenib were diarrhea (50 % vs. 40 %), hypertension (49 % vs. 29 %), weight decrease (37 % vs. 24 %), decreased appetite (29 % vs. 19 %), dysphonia (23 % vs. 10 %), hypothyroidism (21 % vs. 7 %), and upper abdominal pain (16 % vs. 6 %) [42]. Individualized dose titrations of axitinib (5–7 mg and then to 10 mg) in select patients were shown to result in a higher proportion of objective responses (54 % vs. 34 %) as compared to a placebo titration in a recent randomized double-blind phase II trial [43]. The data from these trials establish axitinib as second-line treatment in metastatic RCC with individualized dose titration resulting in a higher rate of objective responses. The optimal method for selecting patients for titration and the scheme by which to titrate require further study.

17.3.6 Cediranib

Cediranib (AstraZeneca) is an oral pan-inhibitor of vascular endothelial growth factor receptors (VEGF-R). In a multicenter, open-label phase II clinical trial, 44 previously untreated patients with mRCC were treated with cediranib 45 mg orally daily, titrated according to tolerance. The primary endpoint of the trial was RECIST-defined objective response (OR). In the 39 patients that were evaluable for response, partial response was observed in 15 (38 %) and stable disease in 18 patients (47 %). Overall tumor control rate was 84 % (95 % CI, 67–95 %). The median PFS was 8.9 months (95%CI, 5.1–12.9). Treatment-related grade 3 or greater adverse events included hypertension (36 %), fatigue (30 %), HFS (16 %), diarrhea (11 %), and anorexia (9 %). Authors concluded that cediranib has substantial antitumor activity in a first-line setting. However, a dose of 45 mg resulted in a higher incidence of grade 3 toxicities or higher and a number of patients had to have dosage adjustments [44].

A double-blind, placebo-controlled study of cediranib in patients with metastatic or recurrent RCC randomized patients 3:1 to cediranib 45 mg/day or placebo. The primary objective was to determine the efficacy judged by changes in tumor size after 12 weeks of therapy. Secondary objectives included assessments of response rate and duration (RECIST), progression-free survival (PFS), and safety. Seventy-one patients were enrolled (cediranib, 53; placebo, 18). The mean percentage change in tumor size between cediranib (−20 %) and placebo (+19 %) was significantly different ($p<0.0001$). Eighteen patients (34 %) in cediranib achieved a partial response and 25 patients (47 %) experienced stable disease. Median PFS was longer in cediranib, 12.1 months, vs. placebo, 2.7 months, including placebo group patients who later received cediranib (HR = 0.45; 90 % CI, 0.26–0.78; $p=0.017$). The most common adverse events with cediranib were diarrhea (88 %), fatigue (66 %), dysphonia (63 %), and hypertension (61 %) [45]. In this trial 43 patients (81 %) had SD or better. Lastly, cediranib is also being evaluated in a phase II single-arm trial for patients with progressive unresectable, recurrent, or metastatic RCC (NCT00227760).

17.3.7 Tivozanib (AV-951)

Tivozanib (AV-951; AVEO Pharmaceuticals, Inc.) is an inhibitor of VEGF-R-1, VEGF-R-2, and VEGF-R-3 as well as c-Kit and PDGF-R. A phase II study showed that AV-951 was active in RCC with an adverse effect profile consistent with that of a selective VEGF-R inhibitor [46, 47]. This was followed by an open-label phase 3 trial (TIVO-1) comparing tivozanib vs. sorafenib in treatment-naïve or cytokine-pretreated patients with advanced clear cell RCC who had a nephrectomy. While PFS was longer in tivozanib (11.9 months vs. 9.1 months), the overall survival

showed a nonsignificant trend toward improved survival in the sorafenib arm (median 29.3 months vs. 28.8 months) [48]. Tivozanib was not approved by the FDA in June 2013 due to inconsistencies in data regarding PFS and OS and an imbalance in post-study treatments, and there are no further development plans in RCC.

17.3.8 Regorafenib (Bay 73–4506)

Regorafenib (BAY 73–4506; Bayer) is an oral multi-kinase inhibitor inhibiting receptors of VEGF, KIT, RET, PDGF, as well as RAF and p38MAPK. Regorafenib 160 mg once daily on 3 weeks on/1 week off was studied in a multicenter, open-label, phase II clinical trial with a primary endpoint of overall response rate. Forty-nine previously untreated patients with predominantly clear cell histology were enrolled in the trial. Objective response was seen in 19 patients (39.6 %, 90 % CI, 27.7–52.5), all of which were partial responses. Drug-related adverse reactions occurred in 48 patients (98 %). These were serious in 17 patients (35 %). The most common grade 3 adverse events were hand–foot syndrome (33 %), diarrhea (10 %), renal failure (10 %), fatigue (8 %), and hypertension (6 %). Two patients had grade 4 AEs: cardiac ischemia or infarction (2), chest pain (1), and hypomagnesemia (1) [49]. Further development plans in RCC are unknown.

17.3.9 Dovitinib (TKI 258)

Dovitinib (TKI25; Novartis) is a TKI that inhibits VEGF and also FGF, PDGF, and TGF receptors. It was recently studied in a phase III multicenter open-label trial where it was compared to sorafenib in patients with clear cell RCC who had been on a previous VEGF-targeted and mTOR therapies. PFS in the dovitinib group was 3.7 months (95 % CI, 3.5–3.9) compared to 3.6 months (95 % CI, 3.5–3.7) in the sorafenib group (HR, 0.86; 95 % CI, 0.72–1.04; one-sided $p=0.063$). Grade 3 or 4 adverse events in the dovitinib group included hypertriglyceridemia (14 %), fatigue (10 %), hypertension (8 %), and diarrhea (7 %). Grade 3 or 4 toxicities in the sorafenib group included hypertension (17 %), fatigue (7 %), and HFS (6 %) [50]. Dovitinib is currently being trialed in a single-arm study in a first-line setting (NCT01791387).

17.4 VEGF-Trap

VEGF-Trap (ziv-aflibercept, Regeneron Pharmaceuticals and Sanofi-Aventis) is a product of the human VEGF-R VEGF-R-1 extracellular immunoglobulin domain 2 and the VEGF-R-2 extracellular immunoglobulin domain 3 fused to human IgG1 Fc molecule. VEGF-Trap thus acts as a soluble decoy receptor to bind VEGF and disrupt subsequent VEGF signaling. VEGF-Trap binds to VEGF with great affinity as well as another angiogenic protein, placental growth factor. In xenograft glioma, rhabdomyosarcoma, and melanoma models, VEGF-Trap-treated mice had significant tumor inhibition of tumor growth and tumor-associated angiogenesis compared with vehicle-treated controls [51, 52].

Two phase I studies with VEGF-Trap have been reported in patients with refractory solid tumors. In the first trial, 30 patients received one (or two) subcutaneous dose(s) of VEGF-Trap followed 4 weeks later by six weekly injections. Drug-related grade 3 adverse events included hypertension and proteinuria without a maximum tolerated dose determined. No objective responses have been observed in this trial [53]. In the second trial, 16 patients have been treated with intravenous VEGF-Trap every 2 weeks. Drug-related grade 3 adverse events included arthralgia and fatigue. One patient with metastatic RCC has maintained stable disease for over 6 months. Objective antitumor activity included a partial response in an advanced ovarian cancer patient and minor responses in metastatic bladder cancer and uterine leiomyosarcoma [54]. Further investigation is ongoing through an Eastern Cooperative Oncology Group trial randomizing metastatic RCC patients resistant to prior sunitinib or sorafenib to one of two doses of VEGF-Trap with a primary endpoint of PFS at 8 weeks (NCT 00357760).

17.5 Newer VEGF-Targeted Agents

Newer VEGF-targeting therapies have currently come to the fore and are the focus of a number of clinical studies. These include newer generation TKIs that target VEGF-R among others as well as monoclonal antibodies that target VEGF-R; these will be discussed briefly.

Linifanib (ABT-869) is an oral potent inhibitor of VEGF-R, PDGF-R, and c-Kit. A recent phase II trial showed clinical activity, but dose modifications had to be made due to toxicities [55]. Brivanib is an oral VEGF-R-2 and FGF-R-1 inhibitor. A phase II, open-label investigation was recently completed to assess its activity in mRCC patients (NCT01253668). Nintedanib (BIBF 120) is an angiokinase inhibitor that targets VEGF-R, FGF-R, and PDGF-R; it is currently being studied in a phase II trial that compares its efficacy and tolerability to sunitinib (NCT 01024920) in a frontline setting. Cabozantinib (XL 184) is a VEGF-R-2 and c-Met inhibitor. It is currently being evaluated in a phase III trial compared to everolimus in patients who had progressive disease after prior VEGF-R tyrosine kinase inhibitor therapy (NCT01865747). Cabozantinib is also being evaluated in a frontline setting in a phase II trial where it is being compared to sunitinib in patients with locally advanced or metastatic RCC (NCT01835158). Ramucirumab (IMC-112-1B) is a monoclonal antibody that targets the VEGF-R. A recent phase 2 study in patients with mRCC who had been on previous TKIs did not reach its primary endpoint of ORR >15 %, but showed that the drug was safe and tolerated well [56].

17.6 Future Directions

Newer therapies in mRCC have greatly expanded therapeutic options. More work needs to be done to determine the ideal sequencing of therapies (VEGF–VEGF vs. VEGF–mTOR) and in personalizing treatments based on toxicities. The combination of VEGF agents or VEGF+mTOR has been shown to be either ineffective or have unacceptable toxicities and is not being further pursued. Additionally, biomarkers that predict response to therapies remain elusive. It is likely that future investigation of these agents will focus on reducing toxicity through alternative scheduling, drug holidays, or other approaches. These agents may also serve as a platform for combination with novel immunotherapeutics being developed in RCC (Table 17.1).

Table 17.1 Summary of select VEGF-targeted agents in the treatment of mRCC

Agent/approach	ORR[a]	Progression-free survival	Comments	FDA approval/ drug status
VEGF receptor inhibition				
Sunitinib	30–45 % in both cytokine-refractory and treatment-naïve patients	11 months (versus 5 months for IFN; $p<0.000001$) in treatment-naïve pts	Overall survival 26.4 months vs. 21.8 months for IFN-treated patients ($p=0.051$)	FDA approved 2006
	8.4 months in cytokine-refractory pts (pooled phase II trial data)		Common toxicity includes fatigue, mucositis, hand–foot syndrome, diarrhea, hypertension, and hypothyroidism	
Sorafenib	2–10 %	5.7 months (vs. 5.6 months in IFN arm; $p=0.5$) in treatment-naïve pts (randomized phase II trial)	Overall survival was 17.8 months vs.15.2 months for patients in the placebo group (hazard ratio, 0.88; $p=0.146$)	FDA approved 2005
		5.5 months (vs. 2.8 months in placebo arm; $p<0.000001$) in cytokine-refractory pts (phase III trial)	Common toxicity includes fatigue, mucositis, hand–foot syndrome, diarrhea, and hypertension	

Table 17.1 (continued)

Agent/approach	ORR[a]	Progression-free survival	Comments	FDA approval/ drug status
Axitinib	44 % (cytokine-refractory RCC)	15.7 months (cytokine-refractory RCC)	Common toxicity includes fatigue, diarrhea, and hypertension	FDA approved 2012
	23 % (sorafenib-refractory RCC)	7.4 months (sorafenib-refractory RCC)	Phase III in frontline refractory RCC versus sorafenib ongoing (AXIS trial)	
Pazopanib	35 %	11.9 months (phase II trial, 69 % without prior treatment)	Phase III trial versus placebo completed and phase III trial versus sunitinib in treatment-naïve RCC are ongoing	FDA approved 2009
Cediranib	34 % PR and 47 % SD	12.1 months vs. 2.7 months in placebo in a phase II trial ($N=71$)	Side effects include fatigue, hypertension, and diarrhea	Not currently approved in RCC
Tivozanib	27 %	11.8 months (in a phase II randomized discontinuation study; $N=272$)	Side effects include hypertension and asthenia	FDA rejection 2013
		12.1 months (in a phase II randomized placebo-controlled trial; $N=111$)		
Regorafenib	39.6 %	Not reported	Side effects include hand–foot syndrome, fatigue, hypertension, mucositis, cardiac ischemia, hypomagnesemia	Not currently approved in RCC
		Based on a phase II open-label trial ($N=49$)		
Dovitinib	4 %	3.7 months vs. 3.6 months in the sorafenib group in a phase III trial	Side effects included hypertriglyceridemia, fatigue, hypertension, and diarrhea	Not currently approved in RCC
VEGF ligand binding				
Bevacizumab	10–13 % as monotherapy	8.5 months in treatment-naïve pts as monotherapy	Common toxicity includes fatigue, anorexia, hypertension, and proteinuria	FDA approved 2009
	26–31 % in combination with IFN	8.5 months and 10.2 months in treatment-naïve pts in combination with IFN		
		4.8 months in cytokine-refractory patients		

Abbreviations: RCC renal cell carcinoma, *ORR* objective response rate, *IFN* interferon alpha
[a]Objective response rate (estimates based on several trials) generally per WHO criteria [57] for hormonal therapy, chemotherapy, and cytokines and per RECIST criteria [58] for targeted therapy

Clinical Vignette

A 55-year-old man with past medical history of hypertension was recently diagnosed with a 10 cm right renal mass invading the left adrenal gland. He is a farmer and denies smoking or alcohol use. He is married and lives with his family in rural Ohio. Imaging studies reveal multiple nodules in both lungs, five of which measure more than 1.5 cm, all worrisome for metastatic disease. On laboratory studies, he has a hemoglobin level of 8.9, a WBC of 7.5 with a normal differential, a platelet count of 300,000, albumin of 4.0, normal serum calcium, normal creatinine, and normal hepatic function tests.

On physical examination, the patient has a Karnofsky performance status of 90 % and has an otherwise normal exam with the exception of a palpable right flank mass. He undergoes a right radical nephrectomy. Pathologic examination confirmed the diagnosis of renal cell carcinoma with clear cell histology, Fuhrman grade 3, with gross extension of tumor to the adrenal gland. There was note of renal vein thrombus. The patient had an uneventful recovery and 4 weeks after the operation is seen in the medical oncology clinic for further evaluation.

Given the diagnosis of metastatic renal cell carcinoma, the need for further treatment is discussed with the patient. Therapeutic options for this patient include the angiogenesis inhibitors bevacizumab (in combination with INF-α), sunitinib, or pazopanib. All of these agents would adversely affect the patient's hypertension, and the TKIs have potential side effects of fatigue, loose stools, and hand–foot skin reaction. Since the patient lives in a rural area, repeated parenteral administration of intravenous bevacizumab + subcutaneous INF-α makes this doublet a less favorable choice. Sunitinib is covered by the patient's insurance plan, and thus, a regimen of 50 mg orally once daily on a 4-week-on, 2-week-off schedule is recommended. Pazopanib would also have been a reasonable choice. The patient was advised to monitor his blood pressure on a daily basis, with a plan to adjust his antihypertensive regimen as needed. Follow-up is scheduled in 4 weeks or earlier if necessary. Restaging imaging scans will be performed after cycle 2 of therapy.

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The Role of mTOR Inhibitors and PI3K Pathway Blockade in Renal Cell Cancer

18

Tanya Dorff and Philip C. Mack

Contents

Key Points

- The mammalian target of rapamycin (mTOR) is a key intermediary of the cellular signal transduction cascade and integrates information about nutrient abundance, cellular energy levels, and growth factor/hormone signaling.
- There are two major mTOR complexes: mTORC1 and mTORC2.
- mTORC1 acts as a signaling intermediary to regulate protein translation. mTORC1 is rapamycin sensitive.
- The current generation of mTOR inhibitors is rapamycin (sirolimus) analogues which block mTORC1 activity by first binding to FK-binding protein 12 (FKBP12), with the resultant complex able to block mTORC1 activity.
- Temsirolimus was US Food and Drug Administration (FDA) approved for use in advanced renal cell carcinoma (RCC) after a 626-patient study showed overall survival improvement for patients with poor-risk features.
- Everolimus was FDA approved for use in patients who progressed after sorafenib, sunitinib, or both after a 417-patient study showed improved progression-free survival compared to placebo.

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_18

- Neither temsirolimus nor everolimus combined with bevacizumab performed better than bevacizumab alone as first-line therapy; monotherapy remains standard.
- Everolimus followed by sunitinib was less effective than sunitinib followed by everolimus; thus, everolimus remains a second- or third-line option.
- Molecular identification of patients most likely to respond is not yet a clinical reality.

18.1 Introduction

The mammalian target of rapamycin (mTOR) is an important intermediary of the signal transduction cascade that reacts to internal and external factors. These factors include nutrient abundance, energy levels, and growth factor/hormone signaling to regulate cellular metabolism, among others. When the mTOR pathway is activated, protein synthesis is stimulated, leading to a diverse array of cellular processes ranging from cell proliferation to cytoskeletal rearrangement.

The mTOR pathway is now an established therapeutic target in oncology, particularly in renal carcinoma where single-agent inhibition of mTOR has improved survival for patients with advanced disease. Current clinical trials of mTOR inhibitors aim to optimize their efficacy through testing of synergistic therapeutic combinations and determining patient subsets, based on tumor molecular profiling, that are most likely to benefit from this class of agents.

The *TOR* gene is highly conserved among eukaryotes [1]. As implied by its name (TOR: target of rapamycin), the gene product was characterized as the putative target of the macrolide rapamycin, a compound derived from a bacterial strain isolated in soil samples from Easter Island [2]. Rapamycin, originally characterized as an antifungal and later as an immunosuppressant, induces cell cycle arrest in eukaryotic cells. In 1991, Heitman and colleagues described two novel genes (named TOR1 and TOR2) that, when mutated, conferred rapamycin resistance in yeast models [3]. In 1994, Brown et al. identified a protein that interacted with the complex formed by rapamycin and the intracellular receptor FKBP12 that was dubbed FRAP (FKBP-rapamycin-associated protein) [4] and demonstrated that its peptide sequences bore significant homology to the yeast TOR1 and TOR2 genes identified by Heitman. Confirmation of their identity was provided by affinity matrix binding experiments performed by Sabers et al. in 1995 using the FKBP12-rapamycin complex as a lure [5].

The mTOR gene encodes a 289 kDal intracellular serine/threonine kinase belonging to the phosphatidylinositol-3-kinase (PI3K)-related kinase (PIKK) family [6, 7]. Toward the amino-terminus, mTOR has tandem HEAT repeats and a FRAP-ATM-TTRAP (FAT) domain. An FKBP12-rapamycin binding (FRB) domain links FAT to the kinase site (Fig. 18.1). In mammalian cells, mTOR is involved in two distinct complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2) [8]. mTORC1 consists of mTOR, mammalian LST8 (mLST8), deptor, and raptor [9–11]. Known substrates for mTORC1 include the proline-rich Akt substrate 40 (PRAS40), 4E-BP1, and p70S6 kinases (S6K1 and S6K2). In mTORC2, the raptor protein is substituted with rictor (rapamycin-insensitive companion of TOR), mSin1 (mammalian SAPK-interacting protein), and Protor1 and includes among its substrates AKT, SGK1, and PKC family members. Rapamycin binds to and inhibits mTORC1, but not mTORC2.

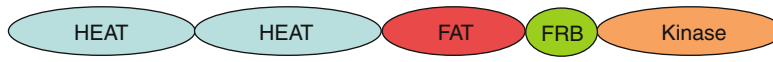


Fig. 18.1 Structure of mTOR. The amino terminal contains tandem HEAT repeats and a FAT domain of unclear function. The kinase domain lies on the carboxy terminal of mTOR and is linked to FAT by FKBP12-rapamycin binding (FRB) domain

18.2 Activity of mTORC1

mTORC1 acts as a sensor and signaling intermediary for nutrient availability, energy levels, and mitogenic growth factors in order to regulate cap-dependent protein translation [1, 12–17]. In essence, mTORC1 functions to ensure that adequate supplies of metabolic precursors as well as positive mitogenic signaling are present prior to cell growth and proliferation. mTORC1 activates the S6 kinases, which subsequently modify the ribosomal protein S6 and the eukaryotic initiation factor 4B (eIF4B), stimulating protein translation. Additionally, mTORC1 suppresses activity of the eIF4E-binding proteins (4E-BPs) via phosphorylation of threonine residues. The 4E-BPs (including 4E-BP1, 2, and 3) function to prevent transcription of eIF4E-dependent mRNAs and formation of key initiation complexes. Thus, when active, mTORC1 deactivates 4E-BPs, releasing eIF4E and enabling the formation of complexes required for initiation of protein synthesis [18–20]. mTORC1 can also bind to PRAS40, which may serve as an inhibitor of mTORC1 by competing with binding to S6K and 4E-BPs, although further elucidation of its role is required. Additional direct activities of mTORC1 may include regulation of lipogenic factors involved in lipid synthesis, as well as inhibition of autophagy and stimulation of mitochondrial biogenesis.

Activity of mTORC1 is governed by both extracellular and intracellular signals (Fig. 18.2). With regard to extracellular activation, mTORC1 responds to receptor-mediated signal transduction cascades initiated through binding of extracellular ligands such as insulin growth factor 1 (IGF-1), epidermal growth factor (EGF), and transforming growth factor (TGF-α) to transmembrane tyrosine kinases triggering their autophosphorylation. Subsequent signaling through the PI3K-Akt cascade results in inhibition of the tuberous sclerosis complexes (TSC1 and TSC2) which in turn release their inhibition of mTORC1 [22]. Akt activation by PI3K is further regulated by the phosphatase and tensin homologue PTEN [23]. Internally, mTORC1 activity can be regulated by hypoxic conditions through REDD1 (regulated in development and DNA damage responses) and energy/nutrient depletion through LKB1-AMPK, either of which can reactivate the mTORC1 suppressors TSC1/TSC2 [24, 25]. mTORC1 is also sensitive to amino levels through Rag GTPase activity ([26] **Smith**).

Components of mTORC1 upstream regulatory pathways are commonly dysregulated in cancer. Loss of PTEN function, through deleterious mutations or promoter methylation [27], or the presence of oncogenic mutations in the PI3K gene leads to constitutive phosphorylation of Akt and mTOR activation. Similarly, mutations in LKB1 and TSC1/2 have been reported [28, 29].

18.3 Activity of mTORC2 and Homeostatic Feedback Loops

Regulation of mTORC2, the alternative protein complex formed by mTOR and rictor, is not well elucidated. This complex is generally considered to be rapamycin *insensitive* in most cell types, although prolonged rapamycin exposure has been reported to impede assembly of the mTORC2 complex in some cases [30]. mTORC2 has been proposed to regulate members of the AGC family of protein kinases, including SGK1 (serum- and glucocorticoid-induced protein kinase 1) involved in ion channel regulation [31]. Intriguingly, mTORC2 upregulates AKT phosphorylation at the Ser473 residue, highlighting the complexity of the mTOR signaling network: while mTOR in the mTORC1 complex is a downstream recipient of AKT signaling, mTOR in the mTORC2 complex is an upstream activator of AKT. Such a relationship may reflect the cellular tendency toward homeostatic correction of signaling imbalances. Further regulatory feedback loops are suggested by the observation that the S6 kinases activated by mTORC1 can repress activity of insulin and insulin-like growth factor (IGF) receptors through degradation of insulin receptor substrate (IRS) proteins which serve as scaffolds for the receptors [32, 33]. This, in turn, reduces receptor-mediated signal transduction through the PI3K-Akt pathway

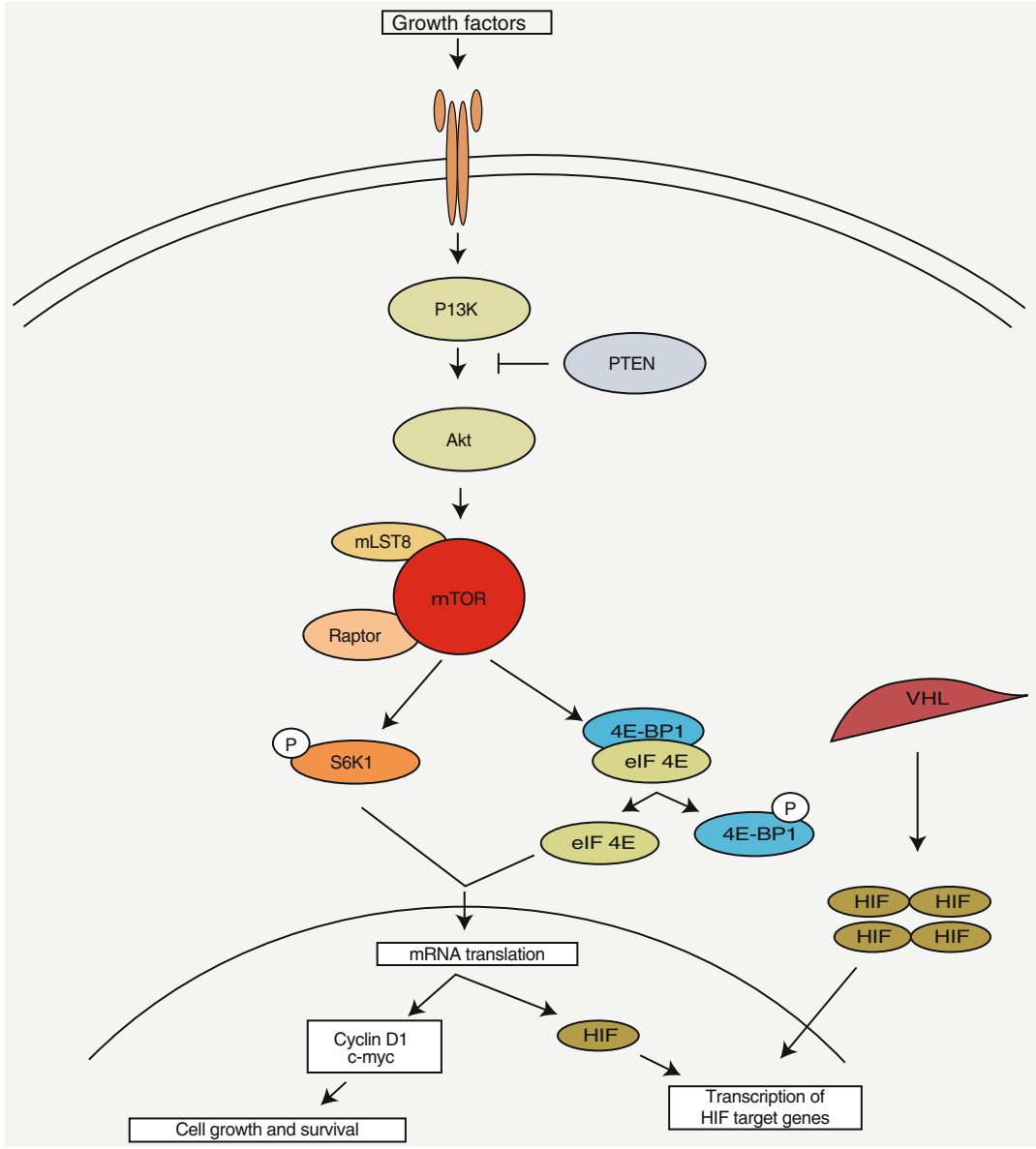


Fig. 18.2 Akt/PI3K/mTOR signaling pathway. mTOR binds to raptor and mLST8 to form mTORC1. MTORC1 is activated by Akt, which is activated by PI3K. PTEN inhibits the activation of Akt by PI3K. Downstream, mTOR phosphorylates p70S6K1 (S6K1) and 4E-BP1 leading to activation of pathways involved in cell growth and survival as well as translation of HIF. In RCC, inactivated VHL is unable to facilitate HIF-α proteolysis leading to HIF accumulation. HIF production is also induced by the activation of the mTOR pathway. The active intranuclear HIF-α induces transcription of HIF target genes such as VEGF and PDGF (Adapted from Rini and Atkins [21])

diminishing mTORC1 activity, completing the negative feedback loop. Similar negative feedback loops involving receptors other than insulin and IGF are thought to be operational (**reviewed in** [34]). A somewhat disconcerting consequence of disrupting these homeostatic processes is that treatment with rapamycin has been shown to *induce* AKT phosphorylation, which may exert oncogenic activity through mTORC1-independent mechanisms.

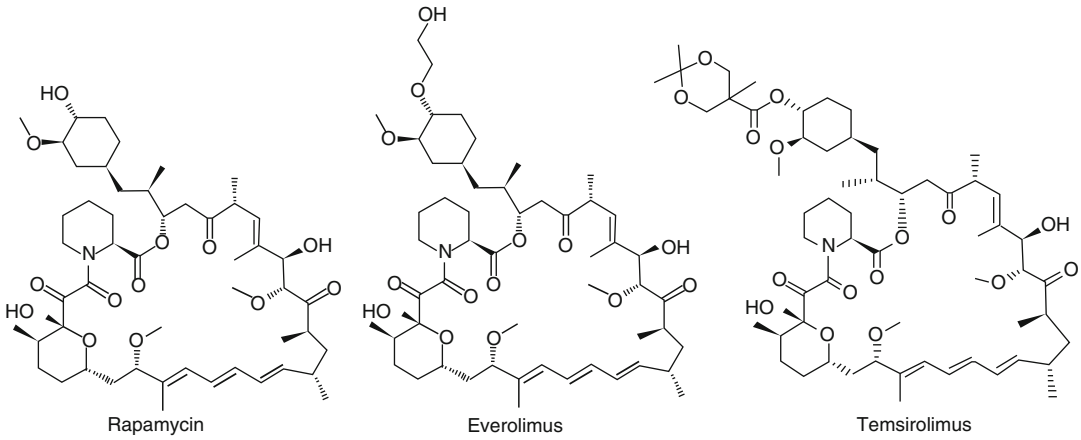


Fig. 18.3 Structure of rapamycin, everolimus, and temsirolimus. Everolimus and temsirolimus substitute the hydroxyl group on carbon 43 in rapamycin by an ether and ester group respectively

18.4 mTOR in RCC

In clear cell carcinoma, the most common histologic subtype of RCC, neoplasia is typically driven by inactivation of the von Hippel-Lindau (VHL) gene [35, 36]. The VHL protein mediates proteasomal degradation of the hypoxia-induced factor (HIF)-1α [37]. When VHL function is disrupted, increased stabilization of HIF-1α results in transcriptional upregulation of genes that promote cell survival and angiogenesis, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-β, and transforming growth factor-α (TGF-α) [38–43]. Further regulation of HIF-1α is achieved by mTOR through the downstream effects of S6K1 and eIF-4E which enhance mRNA translation [21]. Upstream of mTOR, loss of PTEN function has been observed in 20–30 % of RCC tumors [38, 44, 45]. Inhibition of mTOR therefore is likely to decrease angiogenesis in addition to possible direct tumor effect on proliferation and survival.

In an immunohistochemical study, phospho-mTOR staining showed moderate to strong signal in 14 out of 29 clear cell carcinoma specimens, concordant with enhanced phosphorylation of S6K [46]. In a larger study [47] using antibodies against pAkt, PTEN, p27, and pS6 on a tissue microarray constructed from specimens from 375 patients with RCC, the mTOR pathway was found to be more active in clear cell carcinoma, high-grade tumors, and tumors with poor prognostic features.

18.5 mTOR Inhibitors

18.5.1 Rapamycin and Rapamycin Analogues

Rapamycin (sirolimus) is a macrolide secreted by *Streptomyces hygroscopicus*, which was initially isolated from an Easter Island soil sample and reported in 1975 [2, 48]. It was originally described as having antifungal properties with particular activity against *Candida*. Its immunosuppressive [49] properties were later discovered, leading to its wide use in the post-organ transplantation setting [50]. Additionally, it was found to have unique antitumor properties [51, 52].

Rapamycin and its three analogues, temsirolimus, everolimus, and ridaforolimus (formerly deforolimus), have been investigated as possible anticancer agents. These three rapamycin derivatives differ from the original rapamycin molecule at the C43 position through the addition of an ester, ether, or phosphonate group for temsirolimus, everolimus, and ridaforolimus, respectively (Fig. 18.3). Presently, ridaforolimus is at early stages of clinical investigation, while the other analogues have been more extensively studied. Further details are provided in subsequent paragraphs.

18.5.1.1 Temsirolimus

The first mTOR inhibitor to be approved for RCC is temsirolimus (CCI-779), a water-soluble ester analogue of rapamycin. Temsirolimus has been shown to inhibit the growth of normal and cancer cells in vitro [53–56]. Similarly, temsirolimus has been demonstrated to inhibit the growth of various solid tumors including prostate and breast cancer xenografts that are PTEN null and/or Akt overexpressing [57, 58].

Phase I Studies

Dosing

The dosing and safety of intravenous temsirolimus have been investigated in early phase clinical trials in patients with advanced solid tumors [59–62]. The maximum tolerated dose (MTD) with a cyclic dosing regimen (daily for 5 days every 2 weeks) was 15–19 mg/m^2 [63]. In a dose-escalation phase I study, a weekly, 30-min infusion regimen permitted the use of higher doses (7.5–220 mg/m^2) [60]. MTD was not truly achieved, despite the development of thrombocytopenia and reversible rash and stomatitis. In addition, objective partial and minor tumor regressions were seen at doses lower than the MTD. In addition, the variability predicted with flat doses was comparable to body surface area-normalized treatment. Hence, flat dosing was subsequently used for further clinical development [60].

Clinical trials in various advanced cancers thereby used weekly IV doses of 25, 75, or 250 mg [64–66]. The dose needed for optimal biologic activity (i.e., inhibition of mTOR activity) was studied in peripheral blood mononuclear cells [65]. This activity was determined by a decrease in the activity of S6K1, a downstream protein from mTOR, and 25 mg was shown to be sufficient to induce inhibition of this target.

Pharmacokinetics

Absorption Temsirolimus administered intravenously at a dose of 25 mg weekly resulted in a mean maximal drug concentration of 585 ng/mL in whole blood, corresponding to a mean AUC of 1,627 ng h/mL [66]. Its active metabolite, sirolimus, is 40 % bound to lipoproteins in the blood; consequently, elevated plasma lipoprotein levels will increase sirolimus plasma concentrations. In 18 patients with mild to moderate hepatic impairment who received a single dose of sirolimus, the clearance of sirolimus was decreased by more than 30 % [67]; thus, it is recommended that the dose of temsirolimus be reduced to 15 mg weekly in the setting of hepatic impairment.

Metabolism Temsirolimus is metabolized through oxidative hydrolysis to yield sirolimus, the active metabolite [68]. Both temsirolimus and sirolimus are metabolized by the cytochrome P450 (CYP)3A4 pathway to yield several demethylated and hydroxylated isomeric products [69, 70]. Sirolimus is generated 15 min after temsirolimus infusion and reaches a peak at 0.5–2.0 h, followed by a mono-exponential decrease [60]. The concentration of sirolimus is higher than temsirolimus with a mean AUC ratio (sirolimus/temsirolimus) of ~2.5–3.5. When temsirolimus was administered at doses higher than 34 mg/m^2, residual concentrations of sirolimus were noted before the scheduled treatment but did not result in rising concentrations of sirolimus after repeated cycles [60].

Elimination Temsirolimus is excreted predominantly via the feces. When a single 25 mg dose of radiolabeled temsirolimus is administered, 78 % of the radioactivity can be recovered from the feces. Approximately 5 % is recovered in the urine, suggesting a minimal role for renal clearance of temsirolimus. Its mean half-life at a standard dose of 25 mg is approximately 13 h with a total plasma clearance (CL) of 16 l/h. Its active metabolite has a longer half-life with a mean between 61 and 69 h [71] and hence results in higher concentrations than temsirolimus. The clearance is moderate and increases substantially with increasing dose and has a minimal patient intervariability. This is thought to be a result of saturable specific binding of CCI-779 to FKBP in the red blood cell [61].

Tumor Response and Toxicity

In a study on 24 patients with advanced solid tumors, temsirolimus was reported to induce two

confirmed partial responses in patients with breast cancer and RCC. The patient with RCC had documented tumor progression of lung and pleural metastasis on interferon-α and interleukin 2 and received 15 mg/m^2 of temsirolimus [60]. The partial response lasted 6.5 months and was observed after 8 weeks of therapy. Two additional patients with RCC experienced minor tumor regressions after treatment with 15 mg/m^2 and 45 mg/m^2 and had 34 % and 39 % tumor reductions, respectively, with the partial responses lasting for 3 and 4.9 months. In another dose-escalation study on 63 patients with advanced cancers, including 16 patients with RCC, 6 patients had suggestive evidence of clinical benefit, and 2 patients with RCC had unconfirmed partial responses. The first received 3.7 mg/m^2/day of temsirolimus, and the second received 19 mg/m^2/day temsirolimus for 5 cycles and then 15 mg/m^2/day [59]. Three patients had dose-limiting toxicities (stomatitis, vomiting/diarrhea, asthenia, and elevated liver transaminases). Five patients required dose reduction.

Phase II Studies

In RCC, phase II studies have determined the efficacy of temsirolimus monotherapy and combination regimens. Atkins et al. first investigated single-agent temsirolimus on 111 patients with cytokine-resistant RCC [71]. The patients were randomly assigned to weekly treatment with temsirolimus at a dose of 25, 75, or 250 mg. An objective response rate of 7 % (one complete response and seven partial responses) was observed, and 26 % of the patients experienced minor responses. Fifty-one percent of patients overall experienced a partial or complete response or stable disease lasting more than 24 weeks. The median PFS was 5.8 months and the median OS was 15 months. The most common grade 3 or 4 side effects were hyperglycemia (17 %), hypophosphatemia (13 %), anemia (9 %), and hypertriglyceridemia (6 %). Other grade 1 or 2 side effects included maculopapular rash, mucositis, asthenia, and nausea and occurred in more than two thirds of the patients. When these patients were stratified along good-, intermediate-, or poor-risk groups according to the MKCC criteria, OS were 23.8, 22.5, and 8.2 months, respectively. The OS in the poor-risk group was longer than the traditional reported OS of 4.9 months in patients having received IFN [72] and justified further studying in this patient subset.

Another multicenter dose-escalation phase I/II study examined the effect of temsirolimus/IFN combination [73]. An ascending dose (5, 10, 15, 20, or 25 mg) of temsirolimus was administered weekly in combination with IFN (six or nine million units) administered three times per week. Based on dose-limiting toxicities, a dose of 15 mg/6MU was recommended. Among the 39 patients who received the recommended dose, 3 patients achieved partial response and 14 had stable disease for at least 24 weeks, with a median PFS for all patients in the study of 9.1 months. The most common reported grade 3 or 4 side effects included leukopenia, hypophosphatemia, asthenia, anemia, and hypertriglyceridemia.

Phase III Trials

In 2007, the results of the multicenter Global Advanced Renal Cell Carcinoma (Global ARCC) [73] were published. That trial compared temsirolimus to either single-agent IFN or to the temsirolimus/IFN doublet as first-line therapy in patients with "poor-risk" disease. Eligible patients had to have three or more of the following six "poor-risk" features: a serum lactate dehydrogenase level of more than 1.5 times the upper limit of the normal range, a hemoglobin level below the lower limit of the normal range, a corrected serum calcium level of more than 10 mg per deciliter (2.5 mmol per liter), a time from initial diagnosis of renal cell carcinoma to randomization of less than 1 year, a Karnofsky performance score of 60 or 70, or metastases in multiple organs [74, 75]. Eligibility criteria differed from other phase III trials of other targeted therapies by including all histologic subtypes of RCC. The trial also allowed for enrollment of patients with CNS metastases, and patients were not required to have undergone a nephrectomy prior to enrollment. Six hundred twenty-six patients were recruited and randomized to three treatment arms: (1) weekly 25 mg dose of IV temsirolimus weekly ($n=209$), (2) 3 MU interferon alpha (with an escalation to 18 MU or

maximum tolerated dose) subcutaneously three times weekly ($n=207$), and (3) a combination of temsirolimus (15 mg weekly) plus IFN (3 MU with an escalation to 6 MU three times weekly) ($n=210$). Twenty percent of the patients had non-clear cell histology, and 67 % had undergone previous nephrectomy.

The primary end point was overall survival (OS), and the secondary efficacy end points were PFS, the ORR, and the disease control rate for at least 24 weeks. No statistical difference was observed when the combination group and the IFN group were compared with OS of 8.4 and 7.3 months, respectively (HR 0.96, $p=0.70$). However, a prolonged OS of 10.9 months was observed in the temsirolimus monotherapy arm versus 7.3 months in the IFN arm (HR 0.73, $p=0.008$). The objective response rates were not statistically different between the three groups, but more patients in the temsirolimus monotherapy (32.1 %) experienced a clinical benefit compared to the combination group (28.1 %) and IFN monotherapy (15.5 %). An improvement in PFS was also observed ($p<0.001$) in the temsirolimus arm compared to the IFN alone arm, and the reported PFS were 3.8, 1.9, and 3.7 months in the temsirolimus, IFN, and combination arms, respectively. Improvements in OS and PFS were independent of the histological type or the nephrectomy status, although a post hoc analysis suggested that patients with non-clear cell histology (presumably papillary RCC) enjoyed the best reduction in the hazard ratio for death [76, 77].

Patients receiving temsirolimus experienced a higher incidence of hyperglycemia, hyperlipidemia, and hypercholesterolemia compared to patients receiving IFN. They also experienced more rash, stomatitis, and peripheral edema but had a lower incidence of grade 3 and 4 side effects.

The INTORSECT trial compared temsirolimus to sorafenib as second-line therapy. The primary end point of progression-free survival was not significantly different between the two arms, 4.3 months for temsirolimus compared to 3.9 months for sorafenib (two-sided $p=0.19$) [78]. However, overall survival favored sorafenib (HR 1.31 with 95 % CI 1.05–1.63, two-sided $p=0.01$). As such, the highest level of evidence remains for the use of temsirolimus as first-line therapy for poor-risk RCC patients.

Combination Studies

Several phase I studies have evaluated the role of temsirolimus in combination with VEGF-targeted therapy. In the first cohort of a study combining IV temsirolimus 15 mg weekly with oral sunitinib 25 mg daily (4 weeks on, 2 weeks off), there were two DLTs (rash, thrombocytopenia, cellulitis, and gout) in the first cohort of three patients, and the study was deemed not feasible [79]. The same combination of starting doses of sunitinib plus temsirolimus in RCC patients specifically also found grade 3 rash and thrombocytopenia in the first patients and closed early for toxicity [80]. A similar phase I study of temsirolimus plus pazopanib yielded similar conclusions: grade 3 fatigue and electrolyte disturbances, which precluded further dose escalation beyond the first dose level [81]. Combining temsirolimus with sorafenib was found to be tolerable; at a dose of sorafenib 400 mg BID and temsirolimus 15 mg IV weekly, only one of six patients had DLT of mucositis, and this dose has been recommended for phase II testing [82]. Temsirolimus has also been combined with tivozanib, a selective TKI of VEGF receptors 1, 2, and 3 in a population of patients who had mostly been treated with at least one prior targeted therapy for metastatic RCC [83]. The full doses of both agents were tolerated, and promising activity was seen, with a 23 % RECIST partial response rate. Lack of support for tivozanib development in RCC may limit further study of this combination.

Another combination, bevacizumab/temsirolimus, appears to have a better toxicity profile. In two separate abstracts presented at ASCO in 2007 [84] and 2009 [85], Merchan et al. evaluated the safety and efficacy of this combination. In the phase I part involving 12 evaluable patients with stage IV clear cell RCC and who had progressed on up to two previous regimens, 7 patients experienced a PR and 2 patients suffered DLTs (mucositis and hypertriglyceridemia). Following this phase I, a regimen of 10 mg/kg of bevacizumab IV every 2 weeks with temsirolimus 25 mg IV weekly was established. In the

phase II component [85], 35 patients were evaluated. Four patients had PRs and 18 patients had SD, suggesting that 88 % of the patients had experienced clinical benefits. This dosing schedule was moved forward into a phase II trial comparing first-line bevacizumab + temsirolimus (A) against standard sunitinib (B) or bevacizumab + interferon (C) [86] in a 2:1:1 randomization of 171 patients with untreated metastatic RCC. The study was negative, with median progression-free survival of 16.8 months group C, compared to 8.2 months in groups A and B. Toxicity was an issue compromising continued therapy in the combination arm. Temsirolimus plus bevacizumab was also compared to bevacizumab plus interferon as first-line therapy for metastatic RCC in the phase III trial INTORACT. The primary end point of progression-free survival was not met, with median PFS 9.1 months for temsirolimus plus bevacizumab compared to 9.3 months for bevacizumab plus interferon [87]. Finally, the phase II cooperative group trial BeST compared four different treatment regimens in the first-line setting: Bev alone, Bev + Tem, Bev + Sor, and Sor + Tem [88]. The median PFS was 8.7 months for Bev, 7.3 months for Bev + Tem, 11.3 months for Bev + Sor, and 7.7 months for Sor + Tem. The conclusion was that future studies should consider inclusion of selective VEGF inhibitors in combination, and for now single-agent therapy remains standard.

Other combinations of interest combine temsirolimus with agents targeting downstream pathways. A study of nelfinavir plus temsirolimus was performed with the intent of targeting PI3K to overcome resistance to mTOR inhibition. This combination was tolerable but efficacy results are not available [89]. Targeting protein kinase C, a member of a downstream pathway, with bryostatin in combination with temsirolimus resulted in multiple significant PRs in RCC patients [90]. Further study of temsirolimus with such agents may be warranted.

18.5.1.2 Everolimus

Everolimus (RAD-001) was initially developed as an oral immunosuppressive agent as a prophylaxis of rejection in patients who have undergone cardiac, liver, and renal transplants [91, 92]. The dose is 1.5 mg twice daily up to a dose of 6 mg/daily [93, 94]. Everolimus binds to a cytoplasmic protein, of FK-506 binding protein-12, to form a complex that interacts with mTOR. This interaction prevents the phosphorylation of the downstream proteins S6K1 and 4E-BP1 and hence prevents their activation and therefore affects tumor cell metabolism and growth.

In Vitro and Animal Studies

In addition to its immunosuppressant effects, everolimus displays antiproliferative properties against endothelial cells following injury and against tumor cells. In a rat model of renal microvascular injury, everolimus inhibited glomerular endothelial cell proliferation by up to 60 %, an effect that was associated with a reduced phosphorylation of the p70S6 kinase and reduced VEGF levels in the glomeruli. It also inhibits the growth of human-derived cell lines in culture and in xenograft models [95]. In a syngeneic rat pancreatic tumor model, everolimus showed dose-dependent antitumor activity with both daily and weekly administration schedules and statistically significant decrease in the tumor size among the treated subjects of 70–95 % depending on the dose. In this preclinical study, everolimus was well tolerated and had an antitumor potency to that of the cytotoxic agent 5-fluorouracil. Because everolimus also has immunosuppressive effects, it was important to find an adequate therapeutic window. For that purpose, Boulay et al. biochemically profited the mTOR signaling pathway in tumors, skin, and peripheral blood mononuclear cells (PBMCs) and found a decrease in the phosphorylation of 4E-BP1 and inactivation of S6K1 after a single administration of everolimus [95]. This finding suggested that S6K1 from the PBMC could possibly be used as a marker for mTOR inhibition and as a means to assess everolimus treatment schedules in cancer patients.

Phase I Studies

Dosing Schedule

Based on these preliminary findings, a phase I study was conducted by Tanaka et al. [96] to

predict optimal clinical regimens of everolimus. S6K1 from PBMC was used as a marker of mTOR inhibition. A pharmacokinetics/pharmacodynamics model was used to plot the association between everolimus concentrations and level of S6K1 inhibition in PBMCs in both human subjects and rats. A time- and dose-dependent S6K1 inhibition with everolimus was shown. In the rat model, a relationship was shown between S6K1 inhibition and antitumor effect. This model allowed the prediction of PBMC S6K1 inhibition-time profiles in patients receiving everolimus, and a daily administration was found to yield a greater effect than weekly administration at higher doses.

Pharmacokinetics

Absorption Everolimus is administered orally, has a low bioavailability in rats of 10 % [97], but has a fast absorption. The peak everolimus concentration (44.2 ± 13.3 μg/l) is reached within 30 min–1 h after administration with an area under the curve of 219.69 ± μg × h/l [98] and with an approximate half-life of 30 h [99]. The steady state is reached within 7 days. High-fat meals decrease the absorption of everolimus in half [100], and hence the drug should be taken consistently either with food or without. The absorption is possibly also affected by the activity of P-glycoprotein, which reduces the oral bioavailability of drugs that are CYP3A substrates [101]. The protein binding of everolimus is not influenced by moderate hepatic impairment [102].

Metabolism Unlike temsirolimus, everolimus is not degraded to sirolimus but is metabolized essentially in the gut and liver by cytochrome P450 3A4, 3A5, and 2C8 and PgP enzymes into hydroxylated and demethylated metabolites [70, 103]. Hydroxy-everolimus is the most important metabolite, accounting for half of the dose-normalized AUC of the first 24 h (AUC24) of everolimus AUC24. The different metabolites appear within 1.2–2.0 h after administration versus 1.5 h for everolimus [104, 105]. To identify the optimal regimen and dosage of everolimus, O'Donnell et al. (Table 18.1) performed a dose-escalation study on 92 patients with advanced cancer with an everolimus dose range of 5–30 mg/week initially based on transplantation data. However, in view of the preclinical data favoring daily dosing, two regimens of 50 and 70 mg weekly and daily doses of 5 and 10 mg were investigated. S6 kinase 1 activity in PBMC was inhibited for at least 7 days at doses ≥20 mg/week. Evaluation of the stable predose serum trough concentration levels from 26 of the 31 patients treated with the weekly regimen indicated minimal accumulation at all weekly dose levels, with steady state achieved by the second week of treatment. The area under the curve increased proportionally with the dose, but the maximal serum concentration increased less than proportionally at doses ≥20 mg/week. Evaluation of profiles from ten patients on the daily regimen patients showed that a steady-state level was reached within a week. Both maximal serum concentration and AUC increased in a dose proportional manner.

Excretion No definite excretion study has been undertaken, but in patients receiving concurrent cyclosporine radiolabeled everolimus, 80 % of the radioactivity was recovered from the feces, and 5 % was excreted in the urine, after the administration of a 3 mg single dose of everolimus (Everolimus-Summary of Product Characteristics. Novartis Pharma AG, Basel, Switzerland).

Tumor Response and Toxicity

Fifty-five patients were studied by Tabernero in a dose-escalation phase I setting at doses of 20, 50, and 70 mg weekly or 5 and 10 mg daily [106]. A dose- and schedule-dependent inhibition of the mTOR pathway was observed with complete inhibition of pS6K1 and p-eIF-4G at a daily dose of 10 mg or weekly dose of 50 mg or greater. Only two patients had RCC. Clinical benefit was noted in four patients including one patient with RCC who experienced stable disease of 14.6 months on 50 mg/week dose. One patient developed grade 3 stomatitis on the daily dose of 10 mg. On the weekly dose at 70 mg, two patients had grade 3 stomatitis, one had grade 3 neutropenia, and the last developed grade 3 hyperglycemia.

Table 18.1 Dose-escalation studies of temsirolimus and everolimus in patients with advanced RCC

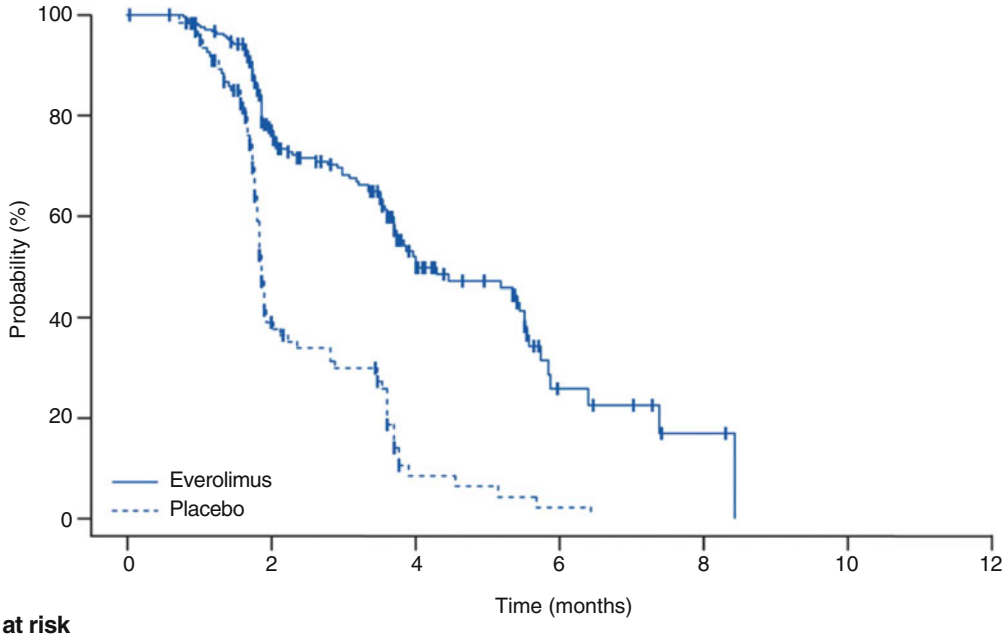
Reference	Year	*n*	Dose	DLT (*n*)	Results
Temsirolimus					
Hidalgo [59]	2006	63	0.75–24 mg/m^2 IV daily × 5 days every 2 weeks	At 19 mg/m^2 DLT in 2 patients (vomiting, diarrhea, ALT)	19 mg/m^2 daily × 5 days every 2 weeks is MTD
Raymond [60]	2004	24	Starting dose 7.5 mg/m^2 IV weekly, escalated up to 220 mg/m^2 IV weekly	At 45 mg/m^2 DLT in 1 patient (grade 3 pancytopenia)	PK analysis suggested flat dosing could be done
				At 220 mg/m^2 DLT in 2 patients (grade 3 stomatitis, ALT elevation)	Two patients with RCC had PR; one at 15 mg/m^2 and one at 45 mg/m^2
Everolimus					
O'Donnell [99]	2008	92 with advanced cancer (10 RCC)	Group 1: weekly dose 5 vs. 10 vs. 30 mg ($n = 18$)	No toxicity	S6K1 activity was inhibited for >7 days at doses higher than 20 mg/week
			Group 2: weekly dose 50 vs. 70 mg ($n = 37$)	Stomatitis and fatigue (1) (50 mg)	Everolimus was tolerated at dosages up to 70 mg/week and 10 mg/day
			Group 3: daily dose 5 vs. 10 mg ($n = 37$)	Hyperglycemia (1) (10 mg)	Five of 10 patients with RCC had PFS ≥6 months
Tabernero [106]	2008	55 with advanced cancer (2 RCC)	Group 1: weekly dose 20 vs. 50 vs. 70 mg ($n = 31$)	70 mg: stomatitis (2) neutropenia (1), hyperglycemia (1)	Complete inhibition of S6K1 and p-eIF-4G at 10 mg/day and ≥50 mg/week
			Group 2: daily dose 5 vs. 10 mg ($n = 24$)	Stomatitis (1) (10 mg)	One of two patients with RCC had stable disease for 14.6+ months at 50 mg weekly

Among the 92 patients evaluated in the phase I trial by O'Donnell, 4 patients experienced partial responses, and 12 patients had a PFS of 6 months or more, including 5 of the 10 patients with RCC. In the two previously described phase I studies, dose-limiting toxicity was seen in one out of six patients [99] receiving everolimus at a weekly dose of 50 mg (stomatitis and fatigue) and four patients receiving 70 mg weekly. Among the patients treated with a daily regimen, one of six patients receiving 10 mg developed hyperglycemia, and another patient also receiving 10 mg developed stomatitis [106].

Phase II Trials

Amato et al. conducted a 2-stage, single-arm, phase 2 trial to determine the PFS of patients with metastatic clear cell RCC receiving everolimus at a daily dose of 10 mg [107]. Forty-one patients were recruited, and 37 patients were evaluable for response. Eligibility criteria included ECOG PS ≤2, satisfactory hematologic, hepatic, renal, and cardiac function. Patients with brain metastases were excluded. The majority of the patients (83 %) had received prior systemic treatment, mostly cytokine therapy with IL-2 and/or IFN-α (61 %). 59 %, 37 %, and 5 % had intermediate, good, and poor risk per MSKCC criteria, respectively.

The results showed a median PFS of 11.2 months and a median OS of 22.1 months. Five patients (14 %) experienced a partial response, and 27 had a stable disease duration longer than 3 months, with 21 (57 %) having a stable disease lasting more than 6 months. More than 70 % of the



Nuber at risk

Everolimus	272	132	47	8	2	0	0
Placebo	138	32	4	1	0	0	0

Fig. 18.4 Kaplan-Meier estimates of progression-free survival by the independent central radiology review (Reproduced from Motzer et al. [108])

patients therefore had partial response or SD >months. The most common grade 1/2 side effects were nausea (38 %), anorexia (38 %), diarrhea (31 %), stomatitis (31 %), pneumonitis (31 %), and rash (26 %). The grade 3/4 side effects included pneumonitis (18 %); transaminase level elevations (10 %); thrombocytopenia, hyperglycemia, and alkaline phosphatase elevations (8 %); and hyperlipidemia (5 %).

Phase III Trials

In view of the phase II results using everolimus as a second-line agent in mRCC, a phase III study was designed to examine the role of everolimus in patients who had progressed on TKIs. The Renal Cell Cancer Treatment with Oral RAD001 Given Daily (RECORD-1), launched in 2005, was a randomized double-blind phase III trial to investigate the role of everolimus in patients who had progressed within 6 months of stopping treatment with sunitinib or sorafenib or both. Four hundred sixteen patients were therefore randomized in a 2:1 ratio to either everolimus at a daily dose of 10 mg ($n=277$) or placebo ($n=139$) with best supportive care. The primary end point was PFS by central review, and the secondary end points included safety, objective response rate, OS, and quality of life. In the study population, 29 %, 56 %, and 15 % of patients had favorable, intermediate, and poor MSKCC risk, respectively, and 97 % of the patients had undergone prior nephrectomy. 44 %, 30 %, and 26 % of patients had received prior sunitinib, sorafenib, or both drugs, respectively, and more than 85 % had received immunotherapy, hormonal therapy, or other treatments.

At the second interim analysis, a significant difference in efficacy between the two study arms was observed, and the trial was therefore stopped after 191 progression events had been observed [108]. A median PFS of 4.0 months was observed in the everolimus group versus 1.9 months in the placebo group (Fig. 18.4). These results prompted the approval of everolimus by the FDA for the treatment of patients with advanced RCC after failure of treatment with sunitinib or sorafenib.

Table 18.2 Phase III trials of temsirolimus and everolimus in patients with advanced RCC

Author and year [citation]	Population	Treatment arms	PFS	OS
Hudes 2007 [74]	$N=626$	Temsirolimus 25 mg IV qwk	3.8 months	10.9 months
	1st line met poor prognosis	IFN 18 mill units SQ TIW	1.9 months	7.3 months
		Tem + IFN 6 mill units SQ TIW	3.7 months	8.4 months ($p=0.008$)[a]
Hutson [78]	$N=512$	Temsirolimus 25 mg IV qwk	4.3 months	12.3 months
	2nd line met (after sunitinib)	Sorafenib 400 mg PO BID	3.9 months	16.6 months ($p=0.01$)
Motzer 2008 [108]	$N=410$	Everolimus 10 mg PO daily	4 months	Not reached
	2nd/3rd line met (after sunitinib, sorafenib, or both)	Placebo	1.9 months ($p<0.0001$)	8.8 months ($p=0.23$)
Motzer 2013 [109]	$N=471$	Everolimus → sunitinib	7.9 months	22.4 months
	1st line met	Sunitinib → everolimus	10.7 months	32 months

[a]OS p value compares Tem to IFN arm

No difference was observed in OS with a median duration of 14.8 months in the everolimus group versus 14.4 months in the placebo group ($p=0.126$). These values however were likely confounded by a crossover effect from the placebo group into the everolimus group. When the confounding factors were accounted for, the corrected OS for crossover was 1.9-fold longer with everolimus compared with placebo only.

The most common side effects during everolimus therapy were stomatitis (44 %), infections (37 %), asthenia (33 %), fatigue (31 %), diarrhea (30 %), cough (30 %), rash (29 %), nausea (26 %), anorexia (25 %), and peripheral edema (25 %). The common grade ¾ side effects (≥5 %) included infections (10 %), dyspnea (7 %), and fatigue (5 %). Four percent of the patients developed pneumonitis, necessitating interruption and/or reduction and corticosteroid use in selected patients.

The RECORD-3 trial asked the question of sequence, comparing PFS for patients randomized to receive everolimus followed by sunitinib at progression compared to sunitinib followed by everolimus at progression [109]. Four hundred seventy-one patients were randomized, and approximately 50 % of randomized patients continued on to the protocol-specified second-line therapy on each arm. Non-inferiority was not achieved, and there was a trend in OS favoring the sequence of sunitinib followed by everolimus, with median OS 22.4 months for everolimus followed by sunitinib, compared to 32 months for sunitinib followed by everolimus (HR 1.24, 95 % CI 0.94–1.64). This confirms the use of everolimus after failure of VEGF TKI as the standard paradigm. Additional exploration of using everolimus earlier in the disease course is ongoing in a cooperative group phase III trial, which will determine whether adjuvant everolimus after nephrectomy in patients with localized RCC increases survival (EVEREST: SWOG S0931; NCT 01120249) (Table 18.2).

Combination Trials

A randomized phase II trial which compared bevacizumab plus everolimus (E + B) to bevacizumab plus interferon (I + B) as first-line therapy for patients with metastatic RCC yielded disappointing results [110]. After randomization of 182 patients to E + B and 183 patients to I + B, the median progression-free survival was not different, 9.3 months compared to 10 months, and median overall survival was also not different, 27.1 months for each arm.

Table 18.3 Combination studies including temsirolimus or everolimus in patients with advanced RCC

Author and year [citation]	Agents combined	Doses	Dose-limiting toxicity	Randomized study outcome
Fischer 2008 [79]	Temsirolimus	15 mg IV qwk	Rash, thrombocytopenia, cellulitis, gout	Not feasible
	Sunitinib	25 mg PO daily		
Patel 2009 [80]	Temsirolimus	15 mg IV qwk	Rash, thrombocytopenia	Not feasible
	Sunitinib	25 mg PO daily		
Semrad 2011 [81]	Temsirolimus	15 mg IV qwk	Anorexia, fatigue, hyponatremia, hypophosphatemia	Not feasible
	Pazopanib	400 mg PO daily		
Patnaik 2007 [82]	Temsirolimus	15 mg IV qwk	Mucositis (1 of 6)	Feasible
	Sorafenib	400 mg PO BID		
Fishman 2013 [83]	Temsirolimus	25 mg IV qwk	None	Feasible
	Tivozanib	1.5 mg PO daily[a]		
Merchan 2007 and 2009 [84, 85]	Temsirolimus	25 mg IV qwk	None	Phase III completed; no advantage over Bev + IFN [79]
	Bevacizumab	10 mg/kg IV q2wk		
Ravaud 2013 [110]	Everolimus	10 mg PO daily	n/a	No advantage over Bev + IFN [110]
	Bevacizumab	10 mg/kg IV q2wk		

[a]Tivozanib is taken 3 weeks on and 1 week off

A trial of the E + B combination as second-line therapy is ongoing, through the Cancer and Leukemia Group B, based on promising interim phase II results. This trial will compare the efficacy of the E + B combination to everolimus plus placebo in patients with mRCC whose disease has progressed after treatment with TKIs. This study is currently recruiting patients with metastatic renal cell carcinoma with at least some clear cell component and has an estimated enrollment of 700 patients. The primary end point will be OS, and the secondary end points will include PFS, ORR, and toxicity.

Another rapamycin analogue, ridaforolimus (AP23573), contains phosphorus and is also being studied as an antineoplastic agent. Ridaforolimus was initially tested in sarcomas [111] with encouraging results. Its combination with capecitabine was recently evaluated in a phase Ib study on 32 patients with multiple advanced solid tumors, including 7 patients with RCC [112]. Two recommend doses of 50 mg or 75 mg weekly were used with capecitabine and were tolerated. One patient with ovarian cancer had a partial response and ten patients experienced stable disease. Unlike temsirolimus and everolimus, the dose used is close to the maximal tolerated dose. Another phase II study has evaluated the ridaforolimus/paclitaxel combination on 29 patients with different cancers, including 1 patient with clear cell carcinoma. The patient with RCC did not respond, but two partial responses were observed in pharyngeal squamous cell and pancreatic carcinoma, and eight patients achieved stable disease ≥4 months [113]. The most common DLT is mucositis, while other mild to moderate side effects include fatigue, nausea, rash, anemia, neutropenia, diarrhea, hyperlipidemia, and thrombocytopenia (Table 18.3).

18.6 Mechanisms of Resistance to mTOR Inhibitors

No durable complete responses have yet been observed with rapamycin analogues in RCC patients, and identifying the mechanisms through which the RCC cells overcome mTOR inhibition will be critical to maximizing their therapeutic impact. Commercially available mTOR inhibitors affect the mTORC1 complex. However, mTORC2 phosphorylates Akt [114] in a positive biofeedback mechanism and hence can limit the

effectiveness of mTOR inhibition. Therefore, agents capable of inhibiting the kinase activity of both mTOR complexes may potentially result in enhanced antineoplastic activity. Mutations affecting mTOR or FKBP12 can lead to an improper attachment to rapamycin and hence are associated with resistance to rapamycin [115–117]. In addition, defects or mutations in downstream effectors such as S6K1 [118, 119] and 4E-BP1 can result in rapamycin resistance [118]. In contrast, activation of the upstream Akt protein appears to induce sensitivity to the mTORi.

Another potential mechanism of resistance involves the IGF receptor (IGFR)/PI3K/Akt pathway disruption. Insulin receptor substrates (IRS) 1 and 2 are activated by IGF-1 and insulin and induce PI3K and mTOR activation. As a downstream protein, S6K1 phosphorylates the IRSs in a negative feedback mechanism and hence decreases the insulin/IGF-1 activation of the PI3K/Akt pathway [33]. Under mTOR inhibition [120–122], this feedback mechanism is lost leading to unopposed IGFR/PI3K/Akt activation. This in turn could possibly decrease the effect of mTORi. Using IGFR inhibitors or inhibitors of PI3K and Akt could help overcome this resistance.

On the other hand, a durable complete remission was seen with everolimus in a patient with urothelial cancer, and genomic sequencing identified a mutation in TSC1 [123]. The finding of sensitivity to everolimus in the setting of TSC1 mutation was further confirmed in an additional group of patients with urothelial cancer from the same clinical trial. Although TSC mutations in sporadic RCC are rare [124], this approach of identifying genetic alterations which confer sensitivity may be another means by which to optimize use of mTOR inhibitors.

Conclusions

mTOR inhibitors are an established class of antineoplastic agents that clearly have unique activity against RCC. Temsirolimus improves survival as a first-line agent in patients with metastatic RCC who have "poor-risk" features. Everolimus improves PFS as a second- or third-line agent and can be used in patients who have progressed on sunitinib, sorafenib, or both. Several ongoing trials will further define the role of these agents in the management of advanced RCC as well as adjuvant therapy following curative resection.

Despite the encouraging results with monotherapy, clinical improvements are fairly modest, and hence sequential and combination treatments are being investigated as a means to improve the therapeutic ratio. Despite the theoretical appeal of combining mTOR inhibitors with VEGF TKI, available data suggest that this paradigm is not clinically feasible due to excess toxicity. While bevacizumab appears to be better tolerated when administered along with an mTOR inhibitor, results of studies using this combination failed to meet their end points. Novel combinations may still be worth investigating, such as mTOR inhibition plus immune therapy.

The approved mTOR inhibitors have improved outcomes in patients with RCC, and we have made strides in identifying optimal treatment strategies in terms of combination and sequence. The next leap forward will come as we continue to identify predictors of sensitivity so that mTOR inhibitors may be applied to the optimal populations of RCC patients.

Clinical Vignette

A 65-year-old gentleman was found by his primary care physician to have anemia. After an unrevealing workup which included gastrointestinal endoscopic studies and a bone marrow biopsy, he had a computed tomography scan of the abdomen which revealed a 9 cm right renal mass and small liver metastases. He underwent a right radical nephrectomy which revealed clear cell renal carcinoma, T4N0.

Shortly after the operation, the patient was found to have progressing liver metastases. He was started on treatment with pazopanib at 800 mg PO daily. After an initial radiographic response which lasted approximately 1 year, he was found to have new and progressing liver metastases.

Treatment with axitinib 5 mg PO BID was then initiated, but the patient had intolerance, and despite dose reduction, the medication had to be discontinued.

He was referred for consideration of additional therapy. He chose to enroll in a randomized clinical trial of everolimus (mTOR inhibitor) versus nivolumab (PD1 inhibitor) and was randomized to everolimus (10 mg PO daily). After 1 month of treatment, he reported mild rash, with grade 1 stomatitis, and diarrhea. These were all managed with appropriate supportive care and he was able to continue on therapy. Restaging scans revealed regression of his systemic disease. He continued on therapy, and up to 1 year later, he continues to have a stable disease. He has had progressive anemia, but otherwise toxicities have been well controlled.

This is a good example of a patient with RCC whose disease has experienced longer than typical disease control with mTOR inhibition after progression on primary VEGF-targeted therapy. Understanding the biology of these individuals may help us discover additional tools for stratification or prediction of response. For now, serial application of available therapies remains the best strategy to prolong progression-free survival as it is not currently possible to predict when and if an individual will respond to a particular treatment.

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19 Combinatorial and Sequential Targeted Therapy in Metastatic Renal Cell Carcinoma

Marc R. Matrana, Bradley J. Atkinson, and Nizar M. Tannir

Contents

Key Points

- The characterization of the VHL-HIF pathway has improved our understanding of RCC pathogenesis and has led to the development of targeted therapies for mRCC, including vascular endothelial growth factor inhibitors (VEGFis) such as tyrosine kinase receptor inhibitors (TKI) and bevacizumab and mTOR inhibitors (mTORis).
- Targeted therapies have largely supplanted cytokine therapies as the treatment of choice for the majority of patients with mRCC.
- Combining targeted therapies may provide more complete blockade of aberrant signaling ultimately leading to additive or synergistic effects and may also have

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_19

the potential to combat resistance that inevitably emerges with single-agent targeted therapies over time.
- Limits to combining targeted therapies include greater toxicities as compared to single-agent therapies.
- Sequential use of targeted therapies has become common practice, allowing for optimal dosing of targeted therapies without the increased toxicity that commonly occurs with combination approaches.
- Targeting different pathways by sequential therapy should help overcome resistance, but research continues to determine the most effective sequence of targeted therapies.
- Novel immune therapies such as anti-PD-1 and anti-PD-L1 agents offer a potentially paradigm shifting approach, but research is currently ongoing to define optimal combinations and/or sequences of these drugs.

19.1 Introduction

Greater insight into the biology of renal cell carcinoma (RCC) has expanded treatment options in metastatic RCC (mRCC). Since 2005, seven targeted agents have been approved by the US Food and Drug Administration (US FDA) for the management of mRCC, but little evidence exists on combining these therapies together or with novel agents, traditional immunotherapies, or chemotherapeutic drugs.

In theory, combining targeted therapies may provide more complete blockade of aberrant signaling ultimately leading to the potential for additive or synergistic effects. Concomitant targeted therapies may also have the potential to combat resistance that inevitably emerge with single-agent targeted therapies over time. Evidence suggests that resistance is mediated by changes which arise within the tumor and in its surrounding microenvironment. Such changes allow for continued proliferation and growth independent of VEGF. It is hypothesized that signaling upstream of receptor blockade could also drive tumor growth independent of usual aberrant proliferative pathways. Hypoxia-inducible factor (HIF), protein kinase B (AKT), and other parallel and upstream pathways likely contribute to resistance [1]. Combination and/or sequential therapy targeting elements independent of classical VEGF pathways may combat resistance, while potentially exhibiting greater efficacy than single-agent therapy. But, despite potential for great disease control in this area, researchers are ultimately challenged by the greater toxicities that have arisen in many trials attempting to combine targeted agents.

Likewise, although sequential therapy with targeted agents following progression on initial treatment is now the standard of care in mRCC, there is only scant evidence on how agents should be used in sequence to optimize treatment following progression on a first-line agent. Here we review the relevant literature and ongoing trials in this area and discuss future opportunities for continued investigation.

19.2 Combination Therapies

19.2.1 Combining Targeted Therapies

Combining approved therapeutic agents in mRCC has been the subject of several research studies to date. The phase III INTORACT (INvestigation of TORisel and Avastin Combination Therapy) trial compared bevacizumab plus temsirolimus versus bevacizumab plus interferon-alpha (IFN-α) in previously untreated patients with mRCC. The median progression-free survival (PFS) in patients treated with temsirolimus/bevacizumab ($n=400$) versus IFN-α/bevacizumab ($n=391$) was 9.1 and 9.3 months, respectively (hazard ratio [HR], 1.1; 95 % CI, 0.9–1.3; $P=0.8$). The median overall survival (OS) (25.8 vs. 25.5 months; HR, 1.0; $P=0.6$) and objective response rates (27.0 % vs. 27.4 %) with temsirolimus/bevacizumab versus IFN-α/bevacizumab were similar. The authors concluded that the combination of an mTOR inhibitor plus bevacizumab was not superior to bevacizumab plus IFN-α frontline treatment in clear cell mRCC [2].

The BeST trial compared single-agent bevacizumab versus combinations of bevacizumab and temsirolimus, bevacizumab and sorafenib, and temsirolimus and sorafenib. The study found that these combinations were not superior to bevacizumab alone and that toxicities were much higher in the combination arms [3].

The Renal Cell cancer treatment with Oral RAD001 given Daily (RECORD-2) study compared combinations of bevacizumab and everolimus to bevacizumab and IFN-α in 365 patients. Outcomes in both arms were similar with an OS of 27.1 months noted in both arms. Remarkably, both treatment combinations were generally well tolerated [4].

Although an earlier trial of sunitinib plus temsirolimus was terminated with only three patients treated due to toxicity, a recent phase I trial reported by Campbell and colleagues incorporated lower doses of each drug. Sunitinib was given at 37.5 mg daily for 2 weeks followed by a 1-week break, and temsirolimus was dose reduced to 8–10 mg weekly [5].

Hainsworth et al. treated 80 patients with mRCC (50 untreated and 30 previously treated) with a combination of bevacizumab and everolimus. They reported a median PFS of 9.1 months in previously untreated patients and 7.1 months in patients previously treated with sunitinib and/or sorafenib. Overall response rates of 30 % in untreated patients and 23 % in previously treated patients were observed. Although the regimen was well tolerated in most patients, serious proteinuria was noted in 25 % of patients, leading to treatment discontinuation in six subjects [6].

Hainsworth and colleagues also tested the combination of bevacizumab and erlotinib, an EGFR inhibitor US FDA approved to treat lung cancer. Preliminary results of this phase II trial showed an objective response rate (ORR) of 25 % in a group of 63 patients with clear cell mRCC. An additional 61 % of patients had either stable disease (SD) or minor therapeutic response. The 1-year PFS was 43 % and treatment was generally well tolerated. Grade 3 toxicities included rash (13 %), diarrhea (13 %), and nausea (10 %) [7].

The combination of bevacizumab and erlotinib was also tested by Bukowski et al. in a randomized phase II study comparing erlotinib combined with bevacizumab to bevacizumab alone in mRCC. A median PFS of 9.9 months in the combination group compared to 8.5 months in the single-agent bevacizumab group (HR, 0.86; 95 % CI, 0.5–1.49; $P = 0.58$) was reported. ORR was 14 % in the combination group versus 13 % in the bevacizumab group. These researchers concluded that the addition of erlotinib to bevacizumab was well tolerated but did not provide additional clinical benefit compared to single-agent bevacizumab [8].

A phase I study conducted by Merchan and colleagues examined the combination of temsirolimus and bevacizumab. They reported 7 patients with PR and 3 patients with SD in 12 evaluable patients [9]. These results led Negrier et al. to study the combination of temsirolimus and bevacizumab in untreated patients with mRCC in the phase II TORAVA trial. They randomized 171 patients (2:1:1) to a temsirolimus and bevacizumab combination (arm A), sunitinib (arm B), or bevacizumab and IFN-α (arm C). Best response rates by RECIST were 25 % in the temsirolimus and bevacizumab combination arm, 24 % in the sunitinib arm, and 34 % in the bevacizumab and IFN-α arm. The researchers found that the toxicity profile of the temsirolimus/bevacizumab combination was higher than expected, with grade III/IV adverse events being reported in 36 % of patients receiving the combination. Two treatment-related deaths were also reported in this cohort. They concluded that there was no evidence to suggest a synergistic or additive effect of this combination [10].

Bevacizumab was combined with sorafenib in a small phase I trial of patients with advanced solid tumors (including three with mRCC). Although one response was noted among the three mRCC patients treated, toxicities were greater than expected and neither drug could be escalated to full dose [11]. A similar phase I study of combination bevacizumab and sorafenib was reported in 14 evaluable patients with mRCC. Responses included four objective PRs and four patients with 20–30 % regression. Only two patients developed PD. Dose-limiting toxicity (DLT) with severe (grade 3) hand-foot syndrome was observed [12].

The combination of bevacizumab with sunitinib has also been investigated. In a phase I trial of 38 patients with advanced solid tumors (including 6 with mRCC), Rini et al. found a decrease in tumor burden in all mRCC patients. Toxicity at

higher dose levels required dose modification [13]. A phase I trial of concurrent bevacizumab and sunitinib in mRCC patients showed a 52 % ORR including one complete response (CR), but the combination was poorly tolerated with a high proportion of patients experiencing toxicity requiring dose modifications and/or study discontinuation [14]. Toxicities included microangiopathic hemolytic anemia, suggesting that excessive blockade of the VEGF pathway may have a more global effect on endothelial viability than desired for antitumor efficacy. In a small case series, Medioni and colleagues reviewed seven patients with mRCC who had disease progression on previous sunitinib monotherapy and were treated with bevacizumab in combination with sunitinib. They noted that two patients had a partial response (PR), four had SD, and one patient had disease progression. The PFS was 8.5 months and OS was 15.1 months [15].

Patel et al. combined temsirolimus and sunitinib in three patients with mRCC. They administered temsirolimus 15 mg IV once weekly and sunitinib 25 mg orally once daily for 4 weeks. Two of the three patients had DLTs requiring discontinuation of treatment (grade 3 rash and grade 3 thrombocytopenia). The third patient experienced mild rash, asthenia, diarrhea, stomatitis, constipation, fever, and rectal hemorrhage. The researchers terminated the study due to these DLTs [16].

Everolimus has also been combined with sorafenib in a small trial by Harzstark and colleagues. These researchers treated 20 mRCC patients with various dose levels of the two drugs in combination. Six patients received everolimus 2.5 mg daily combined with sorafenib 400 mg twice daily, eight patients received everolimus 5 mg daily with sorafenib 400 mg twice daily, and six patients received everolimus 10 mg daily and sorafenib 200 mg twice daily. Everolimus 5 mg daily with sorafenib 400 mg twice daily was established as the maximum tolerated dose. Dose-limiting toxicities included hyperuricemia with gout, pancreatitis, and rash. Treatment-related adverse events occurred in more than 20 % of patients and included diarrhea, hand-foot syndrome, hypertension, hypophosphatemia, hypothyroidism, and rash. Five of the 20 patients treated achieved PR (all 5 had no prior systemic therapy). Seven of eight patients treated with the maximum tolerated dose experienced PR or SD. There was no interaction between everolimus and sorafenib in pharmacokinetic studies [17].

19.2.2 Combining Targeted Agents and Novel Drugs

In addition to combining US FDA-approved targeted agents, researchers are also attempting to combine commercially available targeted agents with novel investigational drugs. Rini et al. tested the combination of sorafenib with AMG 386, a novel Tie2 inhibitor which blocks angiogenesis by sequestering angiopoietin 1 and 2, thus preventing their interaction with the Tie2 receptor on endothelial cells. Previously untreated patients ($n = 152$) with mRCC were randomized 1:1:1 to receive sorafenib 400 mg orally twice daily plus intravenous AMG 386 at 10 mg/kg (arm A) or 3 mg/kg (arm B) or placebo (arm C) once weekly. Patients in arm C could receive open-label AMG 386 at 10 mg/kg weekly plus sorafenib following disease progression. PFS was similar in all 3 arms, whereas ORR was higher in the AMG 386 arms (38 % in arm A, 37 % in arm B, and 24 % in the sorafenib plus placebo group) [18].

The combination of a novel class II histone deacetylase (HDAC) inhibitor, vorinostat, and bevacizumab was tested in patients who had been previously treated with VEGF TKIs by Pili et al. HDAC inhibitors work by inhibiting HIF-1a and have an antitumor effect in combination with VEGFi agents. In this study, 34 patients with clear cell mRCC who had been previously treated with up to 2 prior regimens were treated with vorinostat 200 mg orally twice daily for 2 weeks and bevacizumab 15 mg/kg intravenously every 3 weeks for 21-day cycles. Immunohistochemistry staining was performed on the original nephrectomy specimens. Of 32 patients who were evaluable, 2 experienced grade 4 thrombocytopenia and 3 had grade 3 thromboembolic events. Six objective responses (18 %) were observed, including 1 CR in a patient who had previously been treated with sunitinib with disease progression. Nineteen patients (67 %) had SD. The median PFS was 5.3 months

and OS was 16.2 months [19]. Schreeder and colleagues reported the results of a multicenter phase I trial combining the novel agent perifosine with sorafenib in patients with advanced RCC and other solid tumors. Perifosine (KRX-0401) is a novel oral agent that inhibits Akt activation in the phosphoinositide 3-kinase (PI3K) pathway and affects a number of other signal transduction pathways, including the JNK pathway. They noted SD of more than 12 weeks in six of nine (67 %) evaluable mRCC patients. Median time to progression (TTP) was 26 weeks in this population with a range of 12–62 or more weeks [20].

19.2.3 Combining Targeted Agents and Novel Immunotherapies

Novel immunotherapies, PD-1/PD-L1 inhibitors that target an inhibitory T-cell co-receptor or its ligand (overexpressed by many tumor cells), have promise for improving outcomes in patients with mRCC. Nivolumab (PD-1 Ab, BMS-936558) is the PD-1 inhibitor that is farthest in clinical development for mRCC. It has been studied in a phase III trial in patients who have progressed on prior VEGFi therapy. Results of this trial are awaited [21] (Tables 19.1 and 19.2).

Table 19.1 Selected trials of combination therapies

Combinations	Author, pub date	# of patients	Outcomes
Combinations of targeted agents			
Bevacizumab and everolimus	Hainesworth, 2010 [6]	80	Median PFS 9.1 months in untreated patients, 7.1 months patients previously treated with sunitinib and/or sorafenib. ORR 30 % in untreated patients, 23 % in previously treated patients
Bevacizumab and erlotinib	Hainesworth, 2004 [7]	63	ORR = 25 %. 61 % of patients had SD or minor response. PFS 43 % at 1 year
	Bukowski, 2007 [8]	104 patients (53 bevacizumab, 51 bevacizumab + erlotinib)	Median PFS 9.9 months in combination group vs. 8.5 months in bevacizumab group. ORR 14 % in combination group vs. 13 % in bevacizumab group
Temsirolimus and bevacizumab	Merchan, 2007 [9]	12	7 patients with PR and 3 with SD
	Negrier, 2011 [10]	171 (2:1:1) temsirolimus + bevacizumab, sunitinib, bevacizumab + INF-α	Best response rates by RECIST were 25 % in the temsirolimus and bevacizumab combination arm, 24 % in the sunitinib arm, and 34 % in the bevacizumab and INF-α arm. Major toxicities reported in temsirolimus and bevacizumab combination group
Bevacizumab and sorafenib	Azad, 2008 [11]	3 mRCC	1 response, major toxicities reported
	Sosman, 2006 [22]	14	4 objective PRs, 4 patients with 20–30 % regression, 2 patients with PD
Bevacizumab and sunitinib	Feldman, 207 [14]	26	52 % ORR (1 CR), combination poorly tolerated
	Rini, Garcia, 2009 [23]	6 mRCC	Decreased tumor burden in all mRCC patients
	Medioni, 2009 [15] (retrospective)	7	2 patients with PR, 4 with SD, 1 with PD. PFS 8.5 months and median OS 15.1 months

(continued)

Table 19.1 (continued)

Combinations	Author, pub date	# of patients	Outcomes
Temsirolimus and sunitinib	Patel, 2009 [19]	3	Study terminated early due to toxicities
Everolimus and sorafenib	Harzstark. 2011 [17]	20	5 patients with PR
Combinations of targeted agents with novel drugs			
Sorafenib and AMG 386 (Tie2 inhibitor)	Rini, 2011 [24]	152 (1:1:1) sorafenib + AMG 386 10 mg/kg, sorafenib + AMG 386 3 mg/kg, sorafenib + placebo	PFS similar in all 3 arms, ORR higher in the AMG 386 arms (38 % in sorafenib + AMG 386 10 mg/kg, 37 % in sorafenib + AMG 386 3 mg/kg, and 24 % in the sorafenib + placebo group)
Bevacizumab and vorinostat	Pili, 2010 [25]	34	18 % ORR (1 CR), 67 % with SD
Sorafenib and perifosine	Schreeder, 2008 [26]	9 mRCC	67 % SD, median TTP 26 weeks
Combinations of targeted agents with immunotherapies			
Bevacizumab and IFN-α	Rini and Halabi, 2004 and 2008 [7,18]	732 bevacizumab + IFN-α vs. IFN-α	OS 18.3 months in combination group vs. 17.4 months in the IFN-α group. Median PFS 8.4 months in combination group vs. 4.9 months in IFN-α group
	Escudier et al. 2007 [27]	649 bevacizumab + IFN-α vs. IFN-α + placebo	Median PFS 10.2 months in combination group, 5.4 months in INF-α + placebo group
Sorafenib and IFN-α	Niwakawa et al. 2012 [28]	18	5 PRs, 11 SD
	Ryan, 2007 [29]	62	19 % PR, additional 50 % with unconfirmed PR or SD
	Gollob, 2007 [30]	40	ORR 33 % (95 % CI, 19–49 %), 5 % CR
	Bracarda, 2007 [31]	100	ORR 34.7 % (including 30.6 % with PR and 4.1 % with CR)
	Jonasch, 2010 [32]	80	ORR 30 % (95 % CI, 16.6–46.5 %) in the sorafenib arm, 25 % (95 % CI, 12.7–41.2 %) in combination arm. Median PFS 7.39 months (95 % CI, 5.52–9.20 months) in sorafenib arm, 7.56 months (95 % CI, 5.19–11.07 months) in the sorafenib + IFN-α arm
Gefitinib and IFN-α	Shek, 2011 [12]	21	Median PFS 5.3 (95% CI, 3–10.1) and OS 13.6 months (95% CI, 10.3–NA)
Temsirolimus and IFN-α	Hudes, 2007 [33]	626 temsirolimus vs. IFN-α vs. temsirolimus + IFN-α	Median OS 10.9 months (95 % CI, 8.6–12.7) in temsirolimus group, 7.3 months in IFN-α group (95 % CI, 6.1–8.8), and 8.4 months in combination group (95 % CI, 6.6–10.3)
Sorafenib and IL-2	Procopio, 2011 [4]	128 sorafenib vs. sorafenib + IL-2	Median PFS 33 weeks with sorafenib + IL-2, 30 weeks with sorafenib

Table 19.1 (continued)

Combinations	Author, pub date	# of patients	Outcomes
Combinations of targeted agents with chemotherapy			
Bevacizumab, gemcitabine, and capecitabine	Chung, et al. 2011 [34]	29	24 % of patients with PR, median OS 9.8 months (95 % CI, 6.2–14.9), PFS 5.3 months (95 % CI, 3.9–9.9)
	Pagliario, 2010 [16]	18 patients with sarcomatoid mRCC	Median TTF 5.5 months (95 % CI, 3.7–12+), median OS 12 months (95 % CI, 9.6–24+)
	Jonasch, Lal, 2011 [35] (retrospective)	28	Median PFS 5.9 months, median OS 10.4 months
Sunitinib and gemcitabine	Michaelson, 2008 [36]	9 poor-risk mRCC	5 patients with PR

Table 19.2 Selected sequencing trials

Study/sequence	Author, pub/presentation date	# of patients	Outcomes
RECORD-3/everolimus->sunitinib vs. sunitinib->everolimus	Motzer, ASCO 2013	471	Median PFS 7.9 (5.6–8.2) months for first-line EVE and 10.7 (8.2–11.5) months for first-line SUN. Median OS 22.4 (19.7–NA) months for EVE→SUN and 32.0 (20.5–NA) months for SUN→EVE
AXIS/sunitinib, bevacizumab + IFN-α, temsirolimus, or cytokine (IFN-α or IL-2) ->axitinib vs. sorafenib	Rini, *Lancet*, 2011 [24] and Motzer, *Lancet*, 2013 [37]	723	Median PFS 6.7 months with axitinib compared to 4.7 months with sorafenib. Median OS 20.1 months with axitinib and 19.2 months with sorafenib
Sunitinib->sorafenib vs. Sorafenib->sunitinib	SWTICH	365	Median total PFS 12.5 months in the sorafenib->sunitinib arm and 14.9 months in the sunitinib ->sorafenib arm (HR = 1.01, $P = 0.54$). Median OS 31.5 months in the sorafenib ->sunitinib arm and 30.2 months in the sunitinib->sorafenib arm (HR, 1.00; $P = 0.49$)
GOLD/prior therapy with 1 VEGFi (sunitinib/bevacizumab) + 1 mTORi (everolimus/temsirolimus) either sequence->sorafenib vs. dovitinib	Motzer et al. *Lancet Oncol*, 2014 [38]	570	Median PFS 3.7 months in the dovitinib group and 3.6 months in the sorafenib group (HR, 0.86; 95 % CI, 0.72–1.04; $P = 0.063$). Median OS was 11.1 months and 11.0 months (HR, 0.96; 95 % CI, 0.75–1.22), respectively

Results from some small combination trials of PD-1 or PD-L1 inhibitors have been presented at recent meetings. A trial of nivolumab in combination with sunitinib or pazopanib in pretreated patients showed an ORR of 52 % (17/33) in the nivolumab plus sunitinib arm and 45 % (9/20) in the nivolumab plus pazopanib arm. The PFS rate at 24 weeks was 78 % for nivolumab plus sunitinib and 55 % for nivolumab plus pazopanib [39], but toxicity was substantial, especially in the pazopanib plus nivolumab arm.

Another recent trial by Hammers and colleagues randomized patients with mRCC to receive nivolumab 3 mg/kg + ipilimumab 1 mg/kg (arm N3

Table 19.3 Selected ongoing combination and sequencing trials

	Trial name/description	Primary end point	Clinical trial identifier
Ongoing sequencing trials			
Pazopanib->sorafenib vs. sorafenib->pazopanib	SWITCH-2	PFS	NCT01613846
Sunitinib->temsirolimus	Torisel 404 study	PFS	NCT00474786
Pazopanib->bevacizumab or everolimus	START	TTF	NCT01217931
Everolimus->bevacizumab or pazopanib			
Bevacizumab->everolimus or pazopanib (6 arm study)			
1 or 2 prior VEGFi therapy ->nivolumab vs. everolimus	CheckMate 025	OS	NCT01668784
Ongoing combination trials			
Sunitinib + AGS-003	ADAPT	OS	NCT01582672
CT-011 + DC/RCC fusion vaccine	PD-1 + dendritic cell/ renal cell carcinoma fusion cell vaccine	AEs and ORR	NCT01441765
MEDI4736 + tremelimumab	Anti-PDL1 + anti-CTLA-4	Safety and tolerability	NCT01975831
Nivolumab + anti-LAG-3		Safety and tolerability	NCT01968109
AMP-514 + MEDI4736	Anti-PD-1 +	Safety and tolerability	NCT02118337
Pembrolizumab + PEG-IFN-α vs. pembrolizumab + ipilimumab	Anti-PDL1 + anti-CTLA-4	Safety and tolerability/PFS	NCT02089685
Nivolumab + sunitinib or pazopanib or ipilimumab	Anti-PD-1 + VEGF TKI or anti-CTLA-4	Safety and tolerability	NCT01472081
Nivolumab + ipilimumab vs. sunitinib	CheckMate 214/ anti-PDL1 + anti-CTLA-4	PFS, OS, and ORR	NCT02231749
Pembrolizumab + pazopanib	Anti-PD-1 + VEGF-targeted TKI	Safety and tolerability/PFS	NCT02014636
MPDL3280A+/– bevacizumab vs. sunitinib		PFS	NCT01984242
MK-3475 + axitinib	Anti-PD-1 + VEGF-targeted TKI	Safety and tolerability	NCT02133742
Nivolumab vs. Nivolumab + Bevacizumab vs. Nivolumab + Ipilimumab	Anti-PD1 or Anti-PD1 + Bevacizumab or Anti-PD1 + Anti-CTLA-4	Safety and tolerability	NCT02210117

+ I1) or nivolumab 1 mg/kg + ipilimumab 3 mg/kg (arm N1 + I3) intravenous every 3 weeks for four doses then nivolumab 3 mg/kg every 2 weeks until progression or toxicity. The ORR was 29 % in arm N3 + I1 and 39 % in arm N1 + I3. SD was seen in seven (33 %) patients in the arm N3 + I1 and nine (39 %) patients in arm N1 + I3 [40]; however, the toxicity was higher in the N1 + I3 arm.

Many combination trials pairing PD-1/PD-L1 therapies with other agents are ongoing (see Table 19.3). Most researchers agree that PD-1/PD-L1 inhibition has the potential of completely altering the treatment paradigms in mRCC. But, identifying which patients benefit most from PD-1/PDL-1 inhibitions and which sequences of therapies are most effective will require additional research. The utility of PD-L1 expression, for example, as a predictive biomarker must be defined, and the impact of prior therapies on PD-1/PD-L1 expression will need to be assessed.

19.2.4 Combining Targeted Agents and Traditional Immunotherapies

Several trials have attempted to combine targeted agents with traditional immunotherapies. The

combination of bevacizumab and IFN-α is the only approved combination therapy for the treatment of mRCC. Two phase III trials confirmed the activity of the combination of bevacizumab and IFN-α in patients with clear cell mRCC. In the USA, CALGB 90206 was a two-arm open-label study in which patients with clear cell mRCC without prior systemic therapy were randomized to either bevacizumab plus IFN-α or IFN-α monotherapy. The median PFS in the combination group was 8.4 months versus 4.9 months in the monotherapy group ($P=0.0001$). OS favored the bevacizumab plus IFN-α arm but was not statistically significant (18.3 months for patients in the combination group vs. 17.4 months in the IFN-α monotherapy group; $P=0.097$). Increased fatigue, anorexia, hypertension, and proteinuria were noted in the combination group [23].

In Europe, the AVOREN trial, a blinded and placebo-controlled study, randomized patients with previously untreated mRCC and prior nephrectomy to receive bevacizumab plus IFN-α or IFN-α plus placebo. Median PFS was significantly longer in the bevacizumab plus IFN-α group than in the control group (10.2 months vs. 5.4 months; HR, 0.63; 95 % CI, 0.52–0.75; $P=0.0001$). Median OS was 23.3 months with bevacizumab plus IFN-α and 21.3 months with IFN-α plus placebo (HR, 0.91; 95 % CI, 0.76–1.10; $P=0.3360$). The results of this study supported the approval of combination bevacizumab plus IFN-α for the treatment for mRCC by both the US FDA and the European Medicines Agency (EMEA) [27, 41].

Given the success of combining bevacizumab and IFN-α, several studies have investigated combining other targeted agents with IFN-α.

The combination of sorafenib and IFN-α has been investigated in several trials. In a phase I trial, Niwakawa et al. combined sorafenib with IFN-α. After 2 weeks of single-agent IFN-α, 18 patients were treated with 28-day cycles of continuous sorafenib 200 mg (cohort 1) or 400 mg (cohorts 2 and 3) twice daily combined with intramuscular IFN-α six million international units (mu) (cohorts 1 and 2) or 9 mu (cohort 3) both three times a week. Five patients had confirmed PR and 11 had SD (response rate = 27.8 %). These researchers noted that five patients had dose-limiting toxicities, most commonly fatigue. All 18 patients treated with this combination experienced at least one treatment-related adverse event, including fatigue, fever, cytopenias, weight loss, and decreased appetite [28]. In a phase II study, Ryan et al. evaluated response to sorafenib plus IFN-α in 62 patients. Response rates in the combination therapy group were higher than expected for either drug alone (19 % of patients achieved a PR and an additional 50 % had an unconfirmed PR or SD). Despite high response rates, higher levels of toxicity necessitated dose reductions and limited therapy. The most common toxicities noted included fatigue, anorexia, anemia, diarrhea, nausea, rigors/chills, leukopenia, fever, and transaminase elevation [24]. A similar trial by Gollob and colleagues found comparable results, with a response rate of 33 % (13 of 40 patients) noted. Five percent of patients achieved a CR, but overall increased toxicities led to dose reductions and breaks between cycles [30]. Two different schedules of IFN combined with sorafenib were compared in patients with previously untreated mRCC by Bracarda et al. in the phase II RAPSODY trial. Patients ($n=101$) were randomized to receive sorafenib 400 mg oral twice daily plus subcutaneous IFN, nine million units (MU) three times a week (arm A) or 3 MU five times a week (arm B). The median PFS was 7.9 months in arm A and 8.6 months in arm B ($P=0.049$). In arm A, 9 PRs were observed and 3 CRs and 14 PRs were observed in arm B (17.6 % vs. 34.0 %; $P=0.058$); 24 and 21 patients (47 % and 42 %), respectively, achieved SD. Common grade 3–4 toxicities were fatigue plus asthenia (28 % vs. 16 %; $P=0.32$) and hand-foot skin reactions (20 % vs. 18 %) [42]. Jonasch et al. compared sorafenib versus sorafenib plus IFN-α in 80 patients with mRCC. They reported an ORR of 30 % (95 % CI, 16.6–46.5 %) in the sorafenib arm compared to a 25 % ORR (95 % CI, 12.7–41.2 %) in the combination arm. A median PFS of 7.39 months was observed in the single-agent sorafenib arm (95 % CI, 5.52–9.20 months) versus a PFS of 7.56 months noted in the sorafenib plus IFN-α arm (95 % CI, 5.19–11.07 months). Toxicities were comparable in both arms, leading the researchers to conclude

the outcomes among the two study groups were similar [32].

Shek et al. reported the results of a phase II trial in which they combined gefitinib, an EGFR inhibitor, with pegylated IFN-α, in patients with previously treated mRCC. Twenty-one patients received pegylated IFN-α subcutaneously once weekly (initially 6 μg/kg/week and later reduced to 4 μg/kg/week) for 12 weeks and gefitinib 250 mg orally once daily until disease progression or intolerance. A 6-month PFS rate of 29 % and OS of 13.6 months were reported. The toxicities most commonly noted were myelosuppression, rash, and nausea. The study did not meet the prespecified 6-month PFS rate >50 %, although the authors noted that molecular screenings prior to therapy may identify patients who would benefit from this therapy [26].

In a phase III trial, Hudes et al. randomized patients with previously untreated poor-prognosis mRCC to receive 25 mg of intravenous temsirolimus weekly, 3 mu of IFN-α (with an increase to 18 mu) subcutaneously three times weekly, or combination therapy with 15 mg of temsirolimus weekly plus 6 mu of IFN-α three times weekly. Patients randomized to the temsirolimus-only arm had longer OS (HR, 0.73; 95 % confidence interval [CI], 0.58–0.92; $P=0.008$) than did patients who received IFN-α alone. OS in the combination therapy arm was similar to that of the IFN-α-only arm (HR, 0.96; 95 % CI, 0.76–1.20; $P=0.70$) [33].

Several trials have also attempted to combine targeted agents with interleukin-2 (IL-2). In the ROSORC trial, Procopio et al. randomized 128 patients with mRCC to receive oral sorafenib 400 mg twice daily plus subcutaneous IL-2, 4.5 mu five times per week for 6 out of every 8 weeks, or single-agent sorafenib. After the enrollment of 40 patients, the dose of IL-2 had to be reduced in order to improve tolerability. A median PFS of 33 weeks with sorafenib plus IL-2 compared to 30 weeks with sorafenib alone ($P=0.109$, median follow-up =27 months) was reported. Median PFS for patients receiving the initial higher dose of IL-2 was 43 weeks as compared to 31 weeks for those receiving the lower dose. Common adverse events included hand-foot syndrome, hypertension, and diarrhea. Serious, grade 3–4 adverse events were reported for 38 % of patients receiving combination therapy and 25 % of patients receiving treatment with the single agent. The researchers concluded that combining sorafenib and IL-2 did not significantly improve efficacy, although a trend toward prolonged PFS was associated with the higher dose of IL-2 [25].

A recent phase II study reported by Dandamudi et al. combined bevacizumab with high-dose IL-2 treatment in patients with mRCC. Patients with predominant clear cell histology, measurable disease, Karnofsky performance status of ≥ 80 %, and adequate end-organ function received IL-2 600,000 IU/kg intravenously every 8 h (maximum 28 doses) during two 5-day cycles on days 1 and 15 each 84-day course. In addition, 2 weeks before initiating IL-2, patients began bevacizumab 10 mg/kg infused every 2 weeks. The median PFS reported was 11.2 months (90 % CI, 5.7–17.7 months) with a 2-year PFS of 18 % (90 % CI, 8–27 %). The regimen did not appear to enhance the durable response of single-agent high-dose IL-2. Response rates were at least as high as single-agent therapy, and toxicities did not exceed those expected from each agent alone [43].

19.2.5 Combining Targeted Agents and Chemotherapy

Combining targeted therapy with cytotoxic chemotherapy has also been investigated. The combination of gemcitabine and capecitabine has shown efficacy in mRCC, especially in patients previously treated with immunotherapy or targeted agents [22, 44]. This led researchers to attempt combining gemcitabine and capecitabine with targeted therapies. In a phase II trial, 29 patients received the combination of gemcitabine, capecitabine, and bevacizumab; most patients previously failed treatment with VEGF TKI. Seven patients (24 %) had a PR, with a median PFS of 5.3 months (95 % CI, 3.9–9.9) and median OS of 9.8 months (95 % CI, 6.2–14.9). The regimen was well tolerated, but the trial was ended early because of slow accrual

[45]. Jonasch et al. conducted a retrospective review of patients treated with the combination of gemcitabine, capecitabine, and bevacizumab in patients with clear and non-clear mRCC. Of 28 patients studied, 9 (32.14 %) had clear cell histology, and 10 (35.71 %) had sarcomatoid features. Initial treatment doses consisted of gemcitabine at a mean treatment dose of 786 mg/m^2 every 2 weeks, capecitabine at a mean treatment dose of 2.73 g/day, and bevacizumab at a mean dose of 10 mg/kg every 2 weeks. The authors reported a median PFS of 5.9 months and a median OS of 10.4 months. Among 15 patients who had previous TKI therapy, the median PFS was 6.2 months and median OS was 11.7 months. In patients with sarcomatoid features, median PFS and OS were 3.9 months and 9 months, respectively. Three patients discontinued one or more of the drugs because of adverse reactions [35]. A phase II trial of this combination specifically targeted to patients with sarcomatoid mRCC was reported with 18 patients, 9 of which were alive at last follow-up (median follow-up time was 12.1 months). Five remained on treatment with the gemcitabine, capecitabine, and bevacizumab combination. Dose reductions were required in 12 patients, with common toxicities being hand-foot syndrome (5 patients), fatigue (4 patients), and deep vein thrombosis (2 patients). The estimated median time to treatment failure (TTF) was 5.5 months (95 % CI, 3.7–>12 months) and median OS was 12 months (95 % CI, 9.6–>24 months) [46].

Michaelson et al. reported the results of a phase I trial in which they combined sunitinib with gemcitabine in 34 patients with advanced RCC and other solid tumors. They noted activity of this combination in patients with poor-risk mRCC with five of nine patients achieving a PR [36]. This group has designed a phase II study of this combination in patients with sarcomatoid and/or poor-risk mRCC. This study is ongoing, but not recruiting participants (Combination Sunitinib and Gemcitabine in Sarcomatoid and/or Poor-risk Patients With Metastatic Renal Cell Carcinoma. NCT00556049. http://clinicaltrials.gov/ct2/show/NCT00556049?term=sunitinib+with+gemcitabine&rank=1).

19.3 Sequential Targeted Therapies

Targeted therapies rarely induce complete responses in patients with mRCC; therefore, sequential use of targeted therapies has become common practice to prolong PFS and OS. Although it is not known how best to overcome resistance to targeted therapies, combination or sequential therapy, it is apparent that sequential therapy allows for optimal dosing of targeted therapies without the increased toxicity that commonly occurs with combination approaches. Targeting different pathways by sequential therapy may help overcome resistance that has developed from prior targeted therapy. The most effective sequence of targeted therapies is yet to be determined. However, accumulating evidence supports this current practice for patients with mRCC.

19.3.1 Cytokines and Sequential Targeted Therapies

Prior to targeted therapies, immunotherapy (interleukin-2 and IFN-α) was considered the mainstay systemic treatment for patients with mRCC [47]. Cytokine therapies are associated with substantial toxicity and limited efficacy with ORR ranging from 10 to 23 % and PFS of 3 months depending on dosage and frequency of treatments [48, 49]. High-dose IL-2 is the only US FDA-approved therapy that produces durable CR in approximately 5 % of patients with mRCC; however, patient selection and toxicities limit its use [50]. IFN-α has been the comparator of choice in clinical trials with targeted therapies [51]. No benefit has been seen with sequential second-line cytokine treatment after disease progression on front-line cytokine therapy [52]. Many clinical trials of targeted therapies have been conducted in patients with cytokine-refractory mRCC, thus providing an opportunity to assess the safety and efficacy of sequential use of these therapies.

The phase III randomized, placebo-controlled TARGET (Treatment Approaches in Renal Cancer Global Evaluation Trial) evaluated the

efficacy and safety of sorafenib in patients with advanced RCC that had progressed on systemic therapy [53, 54]. A majority of patients in TARGET had received cytokine therapy prior to enrollment; 83 % of patients on sorafenib and 81 % on placebo received cytokines before enrollment [53]. The median PFS for cytokine-treated patients in the sorafenib arm was 5.5 months compared to 2.7 months in the placebo arm (HR, 0.54; 95 % CI, 0.45–0.64) and was similar in cytokine-naive patients with median PFS of 5.8 months compared with 2.8 months (HR, 0.48; 95 % CI, 0.32–0.73), respectively. A higher incidence of AEs was reported for mRCC patients with prior cytokine therapy (85 % vs. 73 %, respectively). The most frequent drug-related AEs were hand-foot skin reaction (HFSR), rash/desquamation, diarrhea, alopecia, and fatigue [55].

A phase II trial of sunitinib, given on a continuous daily dosing schedule post-cytokine therapy, demonstrated a response rate (RR) of 20 %; in addition, 51 % of patients achieved SD with a median PFS of 8.2 months [56]. The results of two multicenter phase II trials were integrated to assess the efficacy and safety of sunitinib after cytokine therapy [57, 58]. In patients with cytokine-refractory mRCC, the median TTP was 10.7 months; a PR was observed in 33 % of patients and 30 % had SD [57]. Fatigue, diarrhea, stomatitis, HFSR, and hypertension were the most frequently reported AEs in these trials [56, 58].

The efficacy and safety of pazopanib in patients previously treated with cytokines was evaluated in two trials [59–61]. A randomized, double-blind phase III trial by Sternberg and colleagues reported that mRCC patients receiving pazopanib post-cytokine treatment had a median PFS of 7.4 months compared to 4.2 months in those receiving placebo (HR, 0.54; 95 % CI, 0.35, 0.84). The RR in patients receiving pazopanib ($n=135$) was 29 % compared to 3 % in placebo-treated patients ($n=67$) [61]. A phase II randomized discontinuation trial by Hutson and colleagues observed a similar RR of 29.6 % in 71 patients who had prior systemic therapy (89 % cytokine therapy) [59]. Pazopanib was well tolerated in both trials. The most frequent AEs were diarrhea, hypertension, hair color changes, nausea, and fatigue [60, 61].

One retrospective study reviewed the safety of IL-2 (high dose $n=22$ and low dose $n=1$) therapy in mRCC patients who were previously treated with TKIs and/or bevacizumab. Tumor control with subsequent IL-2 treatment was poor with no patients experiencing a CR or PR and only 13 % of patients achieving SD. Only 1 of 23 patients went on to receive a second cycle of IL-2. In addition, 6 of the 15 patients (40 %) who received sunitinib or sorafenib prior to high-dose interleukin-2 experienced severe (grade 3 or 4) cardiac toxicities with one death during IL-2 treatment [62].

19.3.2 Sequential Use of Targeted Therapies

The sequential use of targeted therapies has become standard practice. A survey by Vickers and colleagues of seven cancer centers in the USA and Canada found that in 645 patients with mRCC, 34 % of patients ($n=218$) and 10 % of patients ($n=70$) received 2 and 3 lines of therapy, respectively. Of the 218 patients given second-line therapies, 88 % of patients ($n=192$) were switched to a second VEGFi, including sunitinib ($n=93$), sorafenib ($n=80$), bevacizumab ($n=11$), or axitinib ($n=8$) [63]. This study demonstrates the common practice of sequencing targeted therapies, many of which have similar or overlapping targets. Therefore, it is imperative to evaluate the efficacy and safety of these regimens for the treatment of patients with mRCC.

19.3.3 VEGF Inhibitors: TKIs and Bevacizumab

One retrospective study by Sablin and colleagues reported a combined PFS of 12.5 months with the sequential use of sorafenib and sunitinib in patients with mRCC, first-line sorafenib with a median PFS of 6.0 months and a subsequent PFS of 6.5 months with second-line sunitinib. Initial sorafenib treatment resulted in a 16 % PR rate and a 66 % SD rate. Subsequent sunitinib therapy

resulted in 15 % of patients with PR and 51 % of patients with SD [29]. In a separate retrospective analysis, the sequence of sorafenib followed by sunitinib ($n=29$), sorafenib was associated with a median TTP of 5.1 months, and the sequence regimen was associated with a median TTP of 18.1 months. On frontline sorafenib, 7 % of patients had PR and 62 % of patients achieved SD, after which 21 % of patients had PR and 38 % of patients achieved SD with sequential sunitinib [64]. Overall, this sequence was well tolerated, and there was a trend toward a lower incidence of AEs with the second-line treatment [29, 64].

An open-label phase II clinical trial by Di Lorenzo and colleagues investigated the safety and efficacy of sorafenib in patients with sunitinib-refractory mRCC. The median number of frontline sunitinib cycles received was four (4 weeks on and 2 weeks off per cycle) with 42.3 % of patients achieving an investigator-assessed best response of CR + PR. The majority of patients receiving second-line sorafenib achieved a best response of SD (76.9 %) with few patients achieving a PR (9.6 %). Median TTP and median OS were 16 weeks and 32 weeks, respectively. Treatment was generally well tolerated with most AEs reported as grade 1 or 2 including fatigue, diarrhea, nausea/vomiting, rash, and neutropenia [65]. Similarly, Garcia and colleagues described modest activity with sorafenib in patients with sunitinib- or bevacizumab-refractory mRCC from an open-label, phase II investigation. Patients were permitted to have sorafenib dose escalation up to 800 mg orally, twice daily. The primary outcome, tumor burden reduction of >5 %, was observed in 30 % of patients. However, no RECIST-defined ORR was observed. Patients had a best response of SD (43 %). The median PFS was 4.4 months and median OS was 16 months. No evidence of an improved tumor burden reduction rate or PFS was observed in patients who had sorafenib dose escalation [66].

Sorafenib in the third-line setting after sequential therapy with sunitinib and mTORi was reviewed in a retrospective analysis of 34 mRCC patients. Responses on initial sunitinib therapy included PR in 50 % of patients, 23 % SD, and median PFS of 10 months. Median PFS was 4 months and 2 months and ORR was 12.5 % and 0 % for second-line everolimus or temsirolimus, respectively. Third-line sorafenib achieved a median PFS of 4 months, median OS of 7 months, and overall disease control rate (CR + PR + SD) of 44 %. Grade 3 or 4 AEs were uncommon; however, ten patients required a sorafenib dose reduction [67].

The sequence of sunitinib followed by sorafenib reported by Sablin and colleagues observed a PFS of 5.1 months with sunitinib, followed by a second PFS of 3.9 months with sorafenib. PR was achieved in 23 % of patients and 54 % of patients had SD with sunitinib, after which 9 % of patients achieved PR and 55 % of patients achieved SD with sorafenib [29]. Dudek et al. reported in their retrospective analysis a median TTP of 5.7 months with first-line sunitinib and 8.5 months for the sequence regimen of sunitinib followed by sorafenib. On frontline sunitinib, 5 % of patients achieved PR and 65 % of patients had SD, after which 5 % of patients achieved PR and 30 % of patients had SD with sequential sorafenib [64].

In examining these results, one should be cautioned in drawing conclusions due to the limited sample size and retrospective nature with inherent limitations. Likewise, it should be noted that the median PFS reported herein for first-line sunitinib (range, 5.1–8.3 months) is less than the 11 months observed in a frontline phase III trial [29, 57, 64], and the median PFS for frontline sorafenib (range 5.1–11.5 months) was longer than that reported in untreated patients in a phase II trial (5.7 months) [56, 64, 68, 69].

A recent phase III randomized trial was completed to provide clarity regarding this sequence. The SWITCH trial randomized 365 patients with previously untreated mRCC to receive open-label sorafenib followed by sunitinib (arm A) or sunitinib followed by sorafenib (arm B) at standard doses. There was no statistically significant difference in total PFS across arms (HR, 1.01; $P=0.54$). Likewise, there was no statistically significant difference in OS (HR, 0.997; $P=0.49$) nor in the first PFS across arms (HR, 1.19;

$P=0.92$). Fewer patients crossed over to receive sorafenib ($n=76$) than to receive sunitinib ($n=103$). There was a marked difference in AEs leading to permanent discontinuation between the two groups (18.6/29.5 %). The most frequent side effects under first-line treatment sorafenib compared to sunitinib were alopecia (29/4 %), diarrhea (43/29 %), dysgeusia (8/21 %), fatigue (21/34 %), HFSR (37/20 %), hypertension (24/24 %), nausea (18/24 %), and rash (22/3 %), respectively. AEs were generally lower during second-line therapy [70].

With several other VEGFis in the oncology pipeline, it is important to define the safety and antitumor activity of sequencing regimens other than sorafenib or sunitinib. A phase II study by Rini and colleagues sought to determine the antitumor activity of sunitinib in patients with bevacizumab-refractory mRCC. The median PFS was 7 months with an ORR of 23 % and median OS of 10 months. Toxicities were mostly mild to moderate in severity and included fatigue, hypertension, and hand-foot syndrome [71]. Two large phase III trials evaluating the safety of bevacizumab plus IFN-α collected information about patients' therapy post-protocol. A significant number of patients who received bevacizumab plus IFN-α therapy received a VEGF-targeted agent post-protocol (29 %, $n=96/327$, and 35 %, $n=119/340$). In those patients who received IFN-α alone, 25 % ($n=81/322$) and 48 % ($n=160/332$) received a VEGFi post-protocol. Patients receiving second-line sunitinib had a median OS of 43.6 months, and patients receiving second-line sorafenib had a median OS of 38.6 months as reported by Escudier and colleagues [27]. No direct comparison can be made from these results as sequential treatment was by physician discretion and the patient characteristics (e.g., prognostic scores, performance status, comorbidities) that led to their selection are unknown.

Matrana and colleagues reported the efficacy of pazopanib in patients with treatment-refractory mRCC from a single-center, retrospective analysis. Patients ($n=93$) had received a median of two prior targeted therapies (93 % TKI and 65 % mTORi). The median PFS was 6.5 months (95 % CI, 4.5–9.7 months), and the median OS was 18.1 months (95 % CI, 10.26–NA months). Pazopanib therapy was well tolerated in the salvage setting with few treatment discontinuations due to AEs (12 %) and no treatment-related deaths [72].

A phase II trial by Hainsworth et al. examined second-line treatment with pazopanib after failure of first-line treatment with sunitinib or bevacizumab in patients with mRCC. Fifty-five patients (sunitinib = 39 patients; bevacizumab = 16 patients) received pazopanib 800 mg orally daily. Patients were evaluated for response after 8 weeks of treatment. Fifteen patients (27 %) had an objective response and additional 27 patients (49 %) had SD. The median PFS was 7.5 months (95 % CI, 5.4–9.4 months) and 2-year OS rate was 43 %. Similar PFS was reported with previous treatment of sunitinib or bevacizumab [73].

AXIS, a randomized phase III clinical trial, compared axitinib with sorafenib as second-line therapy in patients ($n=723$) with mRCC. The median PFS was 6.7 months with axitinib compared to 4.7 months with sorafenib (HR, 0.665; 95 % CI, 0.544–0812; $P<0.0001$). Treatment was discontinued because of toxic effects in 4 % of patients treated with axitinib and 8 % of patients treated with sorafenib. Common adverse events were diarrhea, hypertension, and fatigue in the axitinib arm and diarrhea, palmar-plantar erythrodysesthesia, and alopecia in the sorafenib arm [74]. Subsequent analysis found a median OS of 20.1 months (95 % CI, 16.7–23.4 months) with axitinib and 19.2 months (17.5–22.3 months) with sorafenib (HR, 0.969; 95 % CI, 0.800–1.174; $P=0.3744$) [37].

A retrospective analysis has proposed the utility of rechallenging mRCC patients with sunitinib therapy. A review by Zama et al. of 23 patients who had progressed on initial sunitinib therapy and subsequent therapies were rechallenged with sunitinib demonstrated a 22 % PR. The median PFS with initial sunitinib treatment was 13.7 months and 7.2 months with rechallenge. Patients with more than 6-month interval between sunitinib treatments had a longer PFS with rechallenge than patients who started the rechallenge within 6 months (median PFS 16.5 vs. 6.0 months;

$P=0.03$). There were no new significant AEs nor was the severity of prior AEs increased with sunitinib rechallenge [68].

19.3.4 Sequencing Regimens with VEGFi and mTORi

Distinct from TKIs and bevacizumab, the mTORi, everolimus and temsirolimus, interfere with HIF synthesis. Therefore, it is expected that mTORi will have activity in those patients with mRCC refractory to VEGFi.

In the review by Vickers and colleagues, in 24 patients who received an mTORi as second-line therapy after a VEGFi, the TTF was longer in patients who received a VEGFi as second-line therapy compared with an mTORi. It should be noted that a larger proportion of patients who had tumors with sarcomatoid features received a second-line mTORi (13 %) than received a second-line VEGFi (1 %), yet the difference in the TTF between these two groups remained when adjusted for histology [63].

The efficacy of everolimus in mRCC patients who had failed ≤2 prior therapies, one of which was sorafenib or sunitinib, was assessed in a phase II study by Jac and colleagues [75, 76]. The median PFS and OS were 5.5 and 8.0 months, respectively [76]. Similarly, a larger phase III randomized, double-blind, placebo-controlled trial (RECORD-1) of everolimus in patients with mRCC refractory to sunitinib or sorafenib, or both, demonstrated a median PFS of 4.9 months vs. 1.9 months in the everolimus and placebo groups, respectively [77, 78]. Patients were allowed to have received both sunitinib and sorafenib (26 %) and other therapies including bevacizumab and cytokines. Common AEs in both studies included stomatitis, rash, and fatigue [76, 78]. Pneumonitis was identified in 21 patients (8 %) receiving everolimus ($n=272$); 8 patients had grade 3 pneumonitis [78]. Everolimus was approved by the US FDA and EMEA in 2009 for patients with mRCC refractory to sorafenib or sunitinib.

RECORD-3, a randomized phase II trial, compared the sequence of everolimus and sunitinib as frontline therapy in 471 patients with mRCC. The primary objective was to assess PFS non-inferiority of first-line everolimus compared with first-line sunitinib; secondary end points included combined PFS for each sequence, overall survival (OS), and safety. The primary end point was not met; the median PFS was 7.9 months for first-line everolimus and 10.7 months for first-line sunitinib (HR, 1.4; 95 % CI, 1.2–1.8). Among patients who discontinued first line, 108 (45 %) crossed over from everolimus to second-line sunitinib, and 99 (43 %) crossed over from sunitinib to second-line everolimus. The median combined PFS was 21.1 months for sequential everolimus then sunitinib and was 25.8 months for sequential sunitinib then everolimus (HR, 1.3; 95 % CI, 0.9–1.7). The median OS was 22.4 months for sequential everolimus and then sunitinib and 32.0 months for sequential sunitinib and then everolimus (HR, 1.2; 95 % CI, 0.9–1.6). Common adverse events during first-line everolimus or sunitinib were stomatitis (53/57 %), fatigue (45/51 %), and diarrhea (38/57 %), respectively [79]. These results support sunitinib as first-line therapy and everolimus as salvage therapy.

In a retrospective analysis ($n=87$), temsirolimus demonstrated similar efficacy and tolerability in patients with VEGFi refractory mRCC, with median TTP of 3.9 months and median OS of 11.2 months. Patients achieved ORR of 5 % by RECIST criteria, and 65 % of patients achieved SD. The most common grade 3 or 4 AEs included fatigue, rash, and pneumonitis [80]. In another small retrospective analysis of temsirolimus therapy in sunitinib-refractory patients, no grade 3 or 4 AEs were reported [81].

The phase III trial INTORSECT (Investigating Torisel As Second-Line Therapy) compared the efficacy of temsirolimus and sorafenib as second-line therapy in patients with mRCC after disease progression on sunitinib. Patients ($n=512$) were randomized 1:1 to receive temsirolimus 25 mg intravenously once weekly ($n=259$) or sorafenib 400 mg orally twice per day. There was no significant difference between treatment arms for PFS (HR, 0.87; 95 % CI, 0.71–1.07; $P=0.19$) or ORR. Median PFS in the temsirolimus and sorafenib arms was 4.3 and

3.9 months, respectively. However, there was a significant OS difference in favor of sorafenib (HR, 1.31; 95 % CI, 1.05–1.63; $P = 0.01$). Median OS in the temsirolimus and sorafenib arms was 12.3 and 16.6 months, respectively. Safety profiles of both agents were consistent with prior prospective studies [82]. The RECORD-3 trial and INTORSECT trial provide interesting insight into the sequencing of mTORi with VEGFi therapy, in particular sunitinib and sorafenib, respectively. These studies suggest that use of an mTORi is optimally utilized after the development of resistance to VEGFi TKIs [79, 82].

Aberrant pathway activation has been proposed as a mechanism of escape from VEGF-targeted therapies, including fibroblast growth factor (FGF). The GOLD trial (Global Oncologic Learnings for Dovitinib in renal cell carcinoma) is the first phase III trial in which two TKIs have been compared in the third-line setting for patients with mRCC and demonstrated that dovitinib had similar clinical activity to sorafenib in this setting. Patients who had received one previous VEGF-targeted therapy (sunitinib or bevacizumab) plus one previous mTOR inhibitor (everolimus or temsirolimus) in either sequence were randomly assigned to receive open-label dovitinib (TKI that inhibits VEGF, FGF, and platelet-derived growth factor receptors) 500 mg orally, 5-days-on and 2-days-off schedule ($n = 284$) or sorafenib 400 mg orally twice daily ($n = 286$). The median PFS was 3.7 months (95 % CI, 3.5–3.9 months) in the dovitinib group and 3.6 months (3.5–3.7 months) in the sorafenib group (HR, 0.86; 95 % CI, 0.72–1.04; $P = 0.063$); median OS was 11.1 months (95 % CI, 9.5–13.4 months) and 11.0 months (8.6–13.5) (HR, 0.96; 95 % CI, 0.75–1.22), respectively. Common grade 3 or 4 adverse events included hypertriglyceridemia (14 %), fatigue (10 %), hypertension (8 %), and diarrhea (7 %) in the dovitinib group and hypertension (17 %), fatigue (8 %), dyspnea (7 %), and palmar-plantar erythrodysesthesia (6 %) in the sorafenib group. To date, dovitinib has not been approved by the US FDA [38].

19.3.5 Conclusions Regarding Sequential Therapies

Although diverse, these clinical investigations consistently demonstrate disease control with sequential therapies for patients with mRCC. Despite the similarity of their targets, it is apparent that sequential use of these agents does not result in cross-resistance and patients may continue to benefit from second- and third-line therapies. These findings must be confirmed with larger randomized trials. Furthermore, sequential targeted therapies appear well tolerated with AEs similar to that experienced in the frontline setting. Cytokine therapies have a limited role in the sequential setting and may incur greater toxicities [62]. Although the optimal sequence of targeted therapies has not been elucidated, several clinical trials are ongoing to compare sequencing regimens (Table 19.3).

19.4 Future Directions

As understanding of the biological mechanisms underlying development, proliferation, and metastasis progress, newer and more directed targeted therapies are expected. Investigators have sought to apply the principles of cytotoxic chemotherapy that dominated the paradigm of cancer therapeutics in the last decades by combining active agents with nonoverlapping toxicity in order to increase efficacy. This strategy has worked well with cytotoxic agents, but it is clear that targeting one molecular pathway even with multiple targeted agents will not cure mRCC and that therapies which combine agents targeting multiple redundant pathways will be needed. As data emerge about how to appropriately combine targeted agents to maximize efficacy and minimize toxicities, it is likely that combination therapy with multiple agents or with novel agents, which strategically target multiple pathways, will become standard of care.

Likewise, sequential therapies will become more precise, as evidence is published from ongoing and future clinical trials. As data regarding both combination and sequential strategies

emerge, it is also likely that both paradigms will be used together to more accurately and effectively treat mRCC. It is further anticipated that as the various subtypes of mRCC are better defined from a molecular and genetic standpoint, much more specialized and even personalized combinations of sequential therapies will be employed based upon the unique biology of each tumor type, of individual tumors, and even of particular tumor cells in a heterogeneous, quickly mutating cancer. Focus has also shifted to optimizing sequential therapies in order to define which patients respond best to an individual targeted agent or pathway blockade using tissue and circulating biomarkers, with the goal of bringing us closer to truly precision medicine.

Additionally, there is a concerted effort among researchers to better understand the potentially paradigm shifting role of anti-PD-1 and anti-PD-L1 therapies. The efficacy of combining these therapies with novel agents or approved targeted therapies will be evaluated in future trials. Likewise, the optimal sequencing of anti-PD-1 or anti-PD-L1 therapies in combination with targeted agents or anti-CTLA-4 or as monotherapies will have to be addressed in future studies.

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Presurgical Therapy in Renal Cell Carcinoma

20

Eric Jonasch

Contents

Key Points

- Relatively small studies show that presurgical therapy with molecularly targeted agents is relatively safe, although there may be wound healing issues in a subset of patients.
- The currently used agents generally do not result in meaningful downstaging of tumors, although some individuals experience a substantial decrease in tumor size or invasiveness.
- At this point, there are no predictive biomarkers to select patients most likely to benefit from a presurgical strategy.
- Future efforts need to be focused on discovering agents that more effectively downsize and downstage tumors and on finding biomarkers of response.

20.1 Introduction

Cytoreductive nephrectomy was established as a standard of care for patients with metastatic renal cell carcinoma (mRCC) receiving immunotherapy after two studies demonstrated a prolongation of survival in the nephrectomy group [1, 2]. The first of these studies randomized 246 patients between up-front cytoreductive nephrectomy followed by interferon alpha (IFN) therapy versus

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P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_20

IFN alone [1]. The second study had an identical design and randomized 85 individuals [2]. The overall survival (OS) in the larger study was 11.1 months in the nephrectomy plus IFN arm versus 8.1 months in the IFN-only arm. The second study demonstrated a 17-month OS in the nephrectomy arm versus 7 months in the IFN-only arm. Individuals with a performance status of 0 and lung-only disease appeared to gain the largest benefit. A subsequent reanalysis of the data elicited additional prognostic factors [3]. In this study, multivariate analysis indicated that performance status 1 vs. 0 and high alkaline phosphatase were negative prognostic factors, and lung metastasis only was a positive predictor of OS. In patients who survived at least 90 days after randomization, progressive disease within 90 days was a negative prognostic indicator, as was poor performance status.

These studies clearly changed clinical practice for mRCC. Nevertheless, they raise as many questions as they provide answers. First of all, the mechanism of survival prolongation is not known. Secondly, the exact timing of cytoreductive nephrectomy was not explored in these patients. Third, the possibility that there are subsets of patients who are uniquely helped or harmed by a surgical intervention cannot be discounted. And lastly, immunotherapy is being used less frequently today, and we do not know whether the data acquired from immunotherapy based studies is applicable to patients who are receiving molecularly targeted agents.

Bex and colleagues explored the timing of systemic therapy in the context of cytoreductive nephrectomy in a small study published in 2006 [4]. They hypothesized that pretreatment with immunotherapy could be used to select individuals who were most likely to benefit from subsequent cytoreductive nephrectomy. Sixteen patients with metastatic RCC (mRCC) and the primary tumor in place received IFN for 8 weeks. Patients with either partial remission (PR) or stable disease (SD) underwent nephrectomy followed by postoperative IFN maintenance. Eight patients developed either a PR ($n=3$) or SD ($n=5$) at metastatic sites and underwent nephrectomy. Survival at 1 year was 50 % in this patient subset. Eight patients with PD did not undergo surgery and had a median survival of 4 months. A follow-up publication expanded these observations to 33 patients in total [5]. Nephrectomies were not performed in ten (30 %) patients whose cancers demonstrated progression at metastatic sites. Median OS was 4 months in this subset. The median OS of 21 patients with nonprogressive cancer and subsequent cytoreductive nephrectomy was 17 months. The major shortcoming of these studies is the lack of a randomized control population. Critics of this approach could argue that the progressors would have been better off had they undergone up-front nephrectomy. Nevertheless, the data are intriguing and have challenged our established way of thinking about integrating surgery and systemic therapy.

The large majority of patients with mRCC now receive molecularly targeted therapy. How does this therapeutic paradigm shift alter our approach to surgical treatment of this patient population? As of now, we have no phase III data to inform us. In addition, we are faced with several important questions. The first is: how did cytoreductive nephrectomy improve OS survival in patients who received subsequent cytokine therapy, and is this still true today in an environment where most individuals receive molecularly targeted agents? What clinical trials and correlative tools do we need to answer this question? What are we trying to accomplish by treating presurgically? Is it reduction of primary tumor size, of circulating tumor cells, or of established metastases? What types of therapy are best suited for the endpoints outlined above?

20.2 Downsizing and Downstaging

One of the key goals in pretreating patients with a primary tumor in place is to decrease surgical difficulty. To achieve this goal requires true downstaging, with retraction of inferior vena caval thrombus, conversion from radical to partial nephrectomy, or facilitating a

Table 20.1 Clinical trials or case series of patients treated with presurgical or neoadjuvant therapy

Group	*N*	Study type	Treatment	Primary tumor	Disease state
MD Anderson Cancer Center [9]	50	Prospective	Bevacizumab+/– erlotinib	Resectable	Metastatic with primary in place: 50
University of North Carolina [6]	30	Prospective	Sorafenib	Resectable	Metastatic with primary in place: 13 Localized disease: 17
MD Anderson Cancer Center [12]	44	Retrospective	Sunitinib Sorafenib Bevacizumab	Resectable	Metastatic with primary in place: 40 Retroperitoneal recurrence: 4
Cleveland Clinic [8]	19	Retrospective	Sunitinib Sorafenib Bevacizumab	Unresectable	Bilateral primary tumors: 2 Locally advanced: 8 Locally recurrent: 6 Metastatic disease: 3
VU University Medical Center [7]	17	Retrospective	Sunitinib	Mixed	Metastatic with primary in place: 17
Stanford Medical Center [10]	14	Case series	Sunitinib Sorafenib	Mixed	Locally advanced: 2 Metastatic with primary in place: 9 Metastatic site: 3
MD Anderson Cancer Center	24	Prospective	Axitinib	Resectable	Locally advanced: all

Table 20.2 Evidence of surgically significant downstaging

Group	Size change of primary tumor after treatment with targeted therapy	Surgically significant downstaging
MD Anderson Cancer Center [9]	Median 0 % (range +44 to –25)	0/50
University of North Carolina [6]	Median –9.6 % (range +16 to –40 %)	Not determined
MD Anderson Cancer Center [12]	Not reported	Not determined
Cleveland Clinic [8]	Not reported	2/19 patients with bilateral primary tumors were able to undergo a partial nephrectomy plus radical nephrectomy and bilateral partial nephrectomy, respectively
VU University Medical Center [7]	Median –12 % (range +11 to –33 %)	4/17 patients previously not considered surgical candidates underwent nephrectomy
Stanford Medical Center [10]	Median –18 % (range –8 to –25 %)	One patient converted from open to laparoscopic nephrectomy One patient showed regression of IVC thrombus 2 patients with local recurrence were rendered surgical candidates secondary to regression of tumor
MD Anderson Cancer Center	Median –28.3 % (range 5.3–42.9 %)	Five patients underwent partial nephrectomy

laparoscopic as opposed to an open approach. A summary of major studies and reports of patients pretreated with molecularly targeted therapy can be found in Table 20.1. Data on primary tumor shrinkage and downstaging is summarized in Table 20.2.

Cowey and Rathmell reported a 30-patient study evaluating presurgical treatment with sorafenib [6]. Seventeen patients had localized disease and 13 had metastatic disease. After a 1-month course of sorafenib therapy, a median decrease of 9.6 % was observed in primary tumor

size (range 16–40 %), and loss of intratumoral enhancement was observed. According to Response Evaluation Criteria in Solid Tumors (RECIST), two patients had a partial response and 26 had stable disease, with none of the 28 evaluable patients progressing on therapy. A small number of patients experienced true downstaging, resulting in conversion from a planned nephrectomy to a partial nephrectomy in one case and conversion from probable open to a hand-assisted laparoscopic nephrectomy.

Van der Veldt et al. describe a series of 22 patients with primary tumors in place who received sunitinib therapy on an expanded access trial [7]. The decision not to perform a nephrectomy was based on a surgically unresectable primary tumor in ten patients, extensive metastatic burden defined as the sum of the diameter of the metastases exceeding the diameter of the primary tumor in six patients, poor Memorial Sloan-Kettering Cancer Center status in two patients, solitary kidney in two patients, advanced age in one patient, and doctor's choice in one patient. Seventeen patients were evaluable. According to RECIST measurement of the primary tumor, 4 patients had a partial response, 12 had stable disease, and 1 had progressive disease. Concordance between primary and metastatic disease response was seen in 16 of the 17 patients. Three patients ultimately underwent cytoreductive nephrectomy, after substantial primary tumor regression. These patients had been previously considered inoperable because of possible contiguous liver invasion by their primary tumors.

Thomas et al. published a retrospective 19-patient series of individuals treated with targeted therapy and subsequent resection [8]. The indication for neoadjuvant-targeted therapy in patients before primary tumor removal was an unresectable primary tumor or the inability to perform partial nephrectomy in those with bilateral RCC. Eight patients had locally advanced disease, six had a local recurrence, and three had metastatic disease. Two patients had extensive bilateral primary RCC. Twelve patients were treated with sunitinib, three with sorafenib, and four with bevacizumab plus IFN. A median 7.2 % shrinkage was seen across all 19 patients, with a RECIST PR in two primary tumors and a 20 % or greater shrinkage in six other patients. The two patients with extensive bilateral disease achieved successful downsizing of their primary tumors, and underwent partial nephrectomy followed by radical nephrectomy in one case, and bilateral partial nephrectomies in the second case. Eighteen patients underwent open nephrectomy, and three had laparoscopic surgery. One patient (5 %) had a pathological complete response.

Jonasch et al. reported on 50 patients with mRCC and primary tumor in place who received an 8-week course of preoperative bevacizumab followed by cytoreductive nephrectomy [9]. Of 45 radiographically evaluable patients, 22 had some degree of primary tumor growth during the 8-week treatment period, and 13, 7, and 3 experienced a 0–10, 11–20, and greater than 20 % primary tumor shrinkage, respectively. In none of these patients did the change in primary tumor size or characteristics result in a decreased surgical difficulty or a conversion from radical to partial nephrectomy.

Harshman and colleagues reported on 14 patients treated with either sunitinib ($n=10$) or sorafenib ($n=4$) prior to nephrectomy [10]. Presurgical therapy was given with the intention to convert two patients with locally advanced disease to an operative state, downstage nine patients prior to cytoreductive nephrectomy, and three patients prior to metastasectomy. Patients were treated a median of 17 weeks prior to surgery and had a median 2-week washout period. Six of the 11 patients with primary renal masses experienced shrinkage, with median primary tumor shrinkage of 18 % (range 17–25 %).

Karam et al. published the most recent presurgical study [11]. This 23-patient trial tested the impact of axitinib on downsizing primary tumors in patients with biopsy-proven nonmetastatic clear cell RCC. Patients with locally advanced RCC received axitinib 5 mg PO BID for up to 12 weeks. Axitinib was continued until 36 h prior to surgery. Patients then underwent either partial or radical nephrectomy. Median reduction of primary RCC diameter was 28.3 %. Eleven patients experienced a partial response by RECIST. Thirteen patients had stable disease.

The most common side effects included hypertension, fatigue, mucositis, hypothyroidism, and hand-foot syndrome. Postoperatively, two grade 3 and 13 grade 2 complications were noted. No grade 4 or 5 complications were reported in this trial. The impressive results seen in this pilot trial suggest that axitinib may be the most effective cytoreductive antiangiogenic agent in the presurgical setting. Nonetheless, these patients were different from most of the other studies in that they did not have metastatic disease. This may imply that these tumors were less genomically complex and as such may be more responsive to therapy. Follow-up studies in the metastatic presurgical setting are required to confirm the observations seen in this trial.

Despite their small size, these studies provide us with some important information. The first is that pretreatment with antiangiogenic agents does appear to have a modest but consistent downsizing effect on primary tumor size. The second is that there are relatively few instances of downstaging, defined by the switch from a more elaborate or extirpative to a less significant surgery. This could be due to two possibilities. The first is that we are hitting the right target with these agents, but the agents lack potency. The second is that we need to hit either alternate or additional targets with presurgical therapy to see a meaningful change in surgical needs. The recent data with axitinib suggest that it may provide the strongest impact on primary tumors and as such warrants further investigation.

20.3 Safety

One of the major concerns in using antiangiogenic therapy in patients scheduled to undergo an operation is the risk of perioperative complications, delayed wound healing, and wound dehiscence. Major findings from the published studies are summarized in Table 20.3.

Margulis et al. published a retrospective review of perioperative complications in 44 patients treated with a variety of molecularly targeted agents prior to undergoing nephrectomy [12]. Seventeen patients received bevacizumab, 12 received sorafenib, and 15 received sunitinib. These patients were compared to 58 matched controls who did not receive presurgical therapy. A total of 39 complications occurred in 17 (39 %) patients treated with preoperative molecularly targeted therapy and in 20 (28 %) who underwent up-front resection (p 0.287). There were no statistically significant differences in a number of perioperative parameters between patients treated

Table 20.3 Perioperative complications attributable to surgery

Group	Treatment	Number of operations	Perioperative complications attributable to therapy
University of North Carolina [6]	Sorafenib	30	None
MD Anderson Cancer Center [9]	Bevacizumab/erlotinib	42	21 % of patients demonstrated wound healing delays or dehiscence 7 % resulting in treatment delays
MD Anderson Cancer Center [12]	Bevacizumab Sorafenib Sunitinib	44	9.1 % incision related (wound healing delays or secondary dehiscence)
Cleveland Clinic [8]	Sunitinib Sorafenib Bevacizumab	19	16 % of patients (intraoperative hemorrhage during hepatic resection, anastomotic bowel leak, wound seroma, ventral hernia)
VU University Medical Center [7]	Sunitinib	4	None
Stanford Medical Center [10]	Sunitinib Sorafenib	14	Increased incidence of adhesions (86 % of patients)
MD Anderson Cancer Center	Axitinib	23	1 patient with chylous ascites 1 patient with grade 3 postoperative bleeding

with preoperative-targeted molecular therapy and those who underwent up-front surgery. Specifically, only four patients in each group demonstrated any incision-related morbidity. Duration, type, and interval from targeted molecular therapy to surgical intervention were not associated with the risk of perioperative morbidity.

There were very few perioperative complications in the prospective 31-patient presurgical sorafenib study reported by Cowey et al. [6]. No complications of delayed wound healing, surgical dehiscence, or excessive bleeding were observed. One patient had a superficial wound breakdown on postoperative day 8, which responded to conservative management. A second patient experienced a myocardial infarction on postoperative day 1 in the setting of an extensive surgical resection with caval thrombectomy and adrenalectomy.

In the retrospective 19-patient series reported by Thomas et al., perioperative complications were noted in 16 % of patients [8]. One patient had significant intraoperative hemorrhage and disseminated intravascular coagulopathy from a concomitant liver resection. An anastomotic bowel leak and abscess were noted postoperatively in another patient who underwent en bloc resection of a retroperitoneal recurrence and adjacent colon. Two patients (11 %) had minor wound complications, including a wound seroma and a ventral hernia. The higher complication rate in this patient group may be due to a more locally advanced patient cohort in the analysis.

Jonasch et al. reported on complications arising from presurgical bevacizumab therapy in their phase II, 50-patient prospective study [9]. Wound dehiscence resulted in treatment discontinuation for three patients and treatment delay for two others. A total of ten patients had some form of incomplete wound healing at the 4-week postsurgical point, which appeared to be higher than historical controls used for comparison in the study.

In the report by Harshman et al., the 14 patients who underwent presurgical molecularly targeted therapy preoperatively did not experience an increase in perioperative complications [10]. The authors did observe an increased incidence and grade of intraoperative adhesions (86 % vs. 58 %, p=0.001; grade 3 vs. 1, p=0.002) in the treatment group, suggesting an increased level of fibrosis induced by pretreatment. This finding has not been reported by other groups and may be particular to the group of patients and their specific circumstances or may be due to underreporting by other centers. As these patients were treated a median of 17 weeks and there was a median 2-week wait before surgery, duration of treatment or length of washout period may have contributed to these findings.

These data suggest that presurgical treatment with antiangiogenic therapy is relatively safe in patients with RCC. Although direct comparison between small trials is difficult, the 3-week half-life of bevacizumab appears to impact perioperative wound healing more than the oral receptor kinase inhibitors, whose half-life varies between 1 and 3 days (ref: Package inserts). As of now, we do not have any information on the safety of performing surgery in patients pretreated with mammalian target of rapamycin (mTOR) inhibitors.

20.4 Survival

No prospective, randomized studies have yet been published comparing a presurgical approach to standard up-front nephrectomy. The only study with a relatively homogeneous patient population and which provided OS data was the bevacizumab presurgical study by Jonasch et al. [9]. In this study, a patient population which consisted of 81 % intermediate-risk and 19 % poor-risk patients had a median OS of 24.5 months. While many factors can influence an OS endpoint, these data at least suggest that there was no gross diminution of OS in patients treated with a presurgical strategy. Only by performing prospective, randomized studies can the effect of presurgical therapy on OS be elucidated.

20.5 Ongoing Clinical Trials

All of the trials mentioned so far have been either single-arm prospective studies or retrospective reviews. Two randomized studies are

currently underway to address some of the questions posed earlier in this chapter. The first study, named CARMENA (NCT00930033), is addressing the question of whether nephrectomy prolongs survival in patients who receive antiangiogenic therapy. In this 573-patient trial, patients are randomized between up-front nephrectomy followed by standard dose sunitinib therapy and up-front sunitinib therapy alone. The study is powered for equivalence, and the primary endpoint is OS. One of the challenges with this study will be to evaluate outcomes in the context of possible delayed nephrectomy in the nonsurgical arm, which may impact the OS endpoint.

The second study (NCT01099423), supported by the European Organization for Research and Treatment of Cancer (EORTC), randomizes 458 patients between up-front nephrectomy followed by sunitinib therapy versus three cycles of sunitinib followed by cytoreductive nephrectomy in patients deemed appropriate for surgery. This study tests two hypotheses: (1) pretreatment with sunitinib will select those patients who are likely to benefit from cytoreductive nephrectomy, and (2) surgical outcomes will be equivalent or superior after pretreatment because of tumor downsizing and/or downstaging. The primary endpoint of the trial is progression-free survival (PFS) and is powered for superiority of the experimental arm.

20.6 Translational Needs

So far, investigation into risks and benefits of presurgical therapy has been performed in a fairly empirical fashion, with assumptions and hypotheses being formed on the basis of clinical hypotheses and observations. To further refine future clinical trials using presurgical therapy, we need to be able to develop early markers of success. There has been some effort exerted on using imaging as a surrogate marker, and up until now, these efforts have not significantly added to our ability to improve patient care.

There are several methods under development which will be useful in defining early benefit in patients who are receiving molecularly targeted therapy. Measurement of circulating tumor cells will likely provide quantitative and qualitative data on therapeutic response. At the time of publication, existing commercially available platforms are not suitable for use in RCC, but a number of promising strategies are being evaluated. Measurement of circulating cytokines and angiogenesis factors is producing reliable prognostic readouts for several factors, and robust versions of multi-analyte platforms can be deployed in a hypothesis-validating manner [13]. Lastly, single nucleotide polymorphism arrays can provide prognostically significant data from primary tumor biopsies [14] and may be used in the future to predict which treatment has the highest likelihood of benefitting patients.

20.7 Therapeutic Needs

As we further develop the presurgical treatment paradigm, therapies that effectively downstage tumors, kill circulating tumor cells, and eliminate nascent micrometastatic foci are needed to complement improving surgical technique. Candidate agents to downstage tumors will need to impact the epithelial cell directly, and strategies including synthetic lethal screens for candidate molecule(s) may yield interesting leads. Agents that kill circulating tumor cells are needed to leverage the benefit of surgery and prevent a perioperative shower of tumor cells into the circulation from limiting the benefit of cytoreductive nephrectomy. To develop such agents, measurement of circulating tumor cells is required, as outlined in the previous section. Lastly, agents that prevent development of nascent micrometastases, prior to the development of tumor vasculature, will also be of benefit. To develop these agents will require an understanding of how RCC tumor cells interact with other circulating cells, as well as with a microenvironment that has been modified by pretreatment with antiangiogenic or other molecularly targeted therapies.

Conclusions

Presurgical therapy with molecularly targeted agents for patients with mRCC has been demonstrated in multiple clinical trials to be safe and induce some degree of shrinkage of the primary tumor. Whereas some patients have benefitted by having less morbid surgeries, it is difficult to determine whether the majority of patients have benefitted from presurgical therapy. The ongoing EORTC trial randomizing patients between up-front versus delayed nephrectomy may shed some light on this question. Development of biomarkers which precisely measure treatment benefit and disease state will accelerate identification of more appropriate agents to expand the presurgical paradigm.

Clinical Vignette

A 54-year-old woman developed fatigue and abdominal bloating. Imaging was performed demonstrating a large left renal mass, measuring 20 cm, possibly invasive into the left psoas muscle. Biopsy was performed revealing a clear cell RCC with sarcomatoid features. Due to the concerns about resectability, and the ominous histological findings, the patient was treated with frontline sunitinib therapy. After the first cycle of therapy, substantial disease regression was observed. The patient ultimately received a year of treatment and underwent successful resection of her renal mass. Systemic therapy was continued for 4 months postoperatively and was then discontinued. At 12 months post-op, she remains disease-free and is in good health.

This case raises a few key issues. The first is that sarcomatoid histology is associated with a poor overall prognosis, and frequently patients will recur shortly after undergoing nephrectomy, even in the absence of overt systemic disease on imaging studies, and succumb to their cancer. The second is that molecularly targeted agents are capable of downsizing primary tumors, sometimes dramatically. Nevertheless, this is still a fairly rare event, and pretreatment with molecularly targeted agents should be undertaken either if no clear alternatives exist or in the context of a clinical trial. The last point is that we are unsure as to how treatment with molecularly targeted agents interacts with the host microenvironment in the perioperative period. Until we have better data from currently accruing phase III trials, a brief period of postoperative therapy may prevent rapid disease regrowth driven by growth factors expressed during wound healing.

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21 Variant Renal Carcinoma Histologies: Therapeutic Considerations

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Key Points

- Non-clear cell renal carcinomas (NCCRCC) are a diverse set of malignancies with papillary renal cancer being the most common variant, followed by chromophobe and collecting duct.
- Non-clear cell renal carcinomas account for 20–25 % of all renal cancers.

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_21

- Survival of patients with metastatic NCCRCC is variable but especially poor in those with collecting and medullary carcinoma.
- Conventional chemotherapy and immunotherapy have traditionally not been very successful in treating metastatic NCCRCC.
- Sarcomatoid dedifferentiation, which can be seen with any renal cancer histology, portends a poor prognosis, although therapy with doxorubicin and gemcitabine has shown some response.
- Most recent clinical trials of agents in renal cell carcinoma (RCC) have excluded those with non-clear cell histology, and thus there is no standard of care for treatment.
- VEGF pathway inhibition has been examined in multiple retrospective and a few prospective studies in NCCRCC with PRs in the 0–33 % range and SD often seen in >50 %.
- mTOR pathway inhibition also appears promising, especially in papillary renal cancer with SD in >70 %.
- Elucidation of novel pathways specific for NCCRCC subtypes and rational drug development to target those pathways remain important goals.

21.1 Introduction

In 2010, malignant renal tumors were estimated to affect approximately 64,000 individuals in the United States, accounting for 3 % of all malignancies and lead to over 13,000 deaths [99]. Worldwide, in 2008, there were over 270,000 cases of kidney cancer and 116,000 deaths [36]. Malignant renal epithelial tumors account for about 85 % of renal malignancies; of these cancers, approximately 25 % are non-clear cell renal cell carcinoma (NCCRCC). Since therapeutic considerations are ideally tailored to the specific biology and clinical course of a histologic tumor type, this chapter will focus on many of the variant subtypes of NCCRCC. A brief review of the histopathologic, genetic, and molecular features of the most common variant NCCRCC subtypes (summarized in Table 21.1) will be followed by a discussion of survival implications for the various histologies and finally a review of the available data behind therapeutic options for metastatic disease based on the histological subtype (Table 21.2). At this time, there is no set standard of care and a paucity of research for patients with metastatic NCCRCC; however, given that approximately 11 % of metastatic patients fit into this category [53], further investigation is indicated.

21.2 Histopathologic, Genetic, and Molecular Considerations of Non-clear Cell Renal Cell Carcinoma

Detailed pathologic and molecular biologic characteristics of RCC generally and non-clear subtypes specifically are discussed in Chaps. 1 and 2. There are now 14 NCCRCC subtypes identified [59, 102]. Briefly, the major non-clear subtypes include [59]:

21.2.1 Papillary Carcinoma (PRCC)

Papillary carcinoma (PRCC) is thought to arise from either the proximal or distal convoluted tubules of the nephron and accounts for 10–15 % of localized RCC in most large series [13, 71, 80]. In approximately 10 % of PRCC cases, tumors are multifocal or bilateral [101]. Two morphologic subtypes of PRCC, type 1 with small cells and little cytoplasm and type 2 with large cells and eosinophilic cytoplasm, have been identified and shown to have different genetic profiles (Fig. 21.1a, b) [2, 25, 52, 71, 90]. Further work has suggested two separate molecular classes of PRCC: the first class, exhibiting excellent survival, has dysregulation of G1-S checkpoint genes and higher c-MET expression and combines morphologic type 1, low-grade type 2, and mixed type 1/low-grade type 2 tumors, and the second class, exhibiting poor survival, has dysregulation of G2-M checkpoint genes and is morphologically composed of high-grade type 2 tumors [129]. Although hereditary PRCC is associated with

Table 21.1 Most common non-clear cell renal cell carcinoma subtypes

Type	Prevalence/histology	Genetic alterations	Pertinent molecular pathways
Papillary RCC type 1 and 2	10–15 % Type 1 with small cells and little cytoplasm; basophilic Type 2 with large cells and eosinophilic cytoplasm; hypovascular	Trisomy of chromosome 7 and 17/del Y in type 1 Multiple cytogenetic abnormalities in type 2	Dysregulation of G1-S checkpoint genes and G2-M checkpoint genes c-MET proto-oncogene-activating mutation leading to constitutive activation of the hepatocyte growth factor (HGF)/MET pathway VEGF and VEGFR expression
Chromophobe RCC	4–10 % Large polygonal cells with transparent or reticulated cytoplasm Eosinophilic variant with purely eosinophilic cells	LOH at multiple chromosomes Hypodiploid DNA content	VEGF and upregulation of c-KIT has been noted in tumor specimens In familial chromophobe RCC, a tumor suppressor, folliculin, has been identified and may be associated with the mTOR pathway
Collecting duct RCC (Bellini duct)	0.5–2 % Irregularly angulated glands Desmoplastic stroma	Monosomies LOH of 8p, 13q, 1q, 6p, 9p, 21q	High incidence of c-ErbB-2 oncogene amplification
Renal medullary carcinoma	Less than 1 % Poorly differentiated with rhabdoid elements Eosinophilic with clear nuclei	Rare 22q11.2 inactivation (INI1/hSNF5 tumor suppressor)	BCR and ABL gene amplification without BCR-ABL translocation Topo II overexpression
Xp11 translocation carcinoma	Less than 2 % Both clear cells and papillary architecture Psammoma bodies	Various translocations of Xp11.2	Translocations of chromosome Xp11.2 leading to gene fusions of transcription factor E3

Table 21.2 Targeted treatment options for NCCRCC

Type	Study	N/agent	Relevant outcomes
NCCRCC not further subclassified or multiple subtypes included in study	Rowinsky et al. (2004) [94]	14/panitumumab	14 % PR; 43 % SD; PFS of 92 days
	Ronnen et al. (2006) [93]	~12/bortezomib	1 CR in a RMC patient
	Dutcher et al. (2009) [29]	~120/temsirolimus	OS 11.6 months; PRCC in ~75 %; CHRCC in ~10–15 %
	Gore et al. (2009) [39]	437/sunitinib	ORR 11 %; 57 % SD
	Plimack et al. (2009) [85]	6/BRYO	At least 1 PR seen
	Molina et al. (2012) [72]	22/sunitinib	5 % PR; 71 % SD; PFS 5.5 months
	Grünwald et al. (2012) [40]	75/everolimus	Median treatment duration 12 weeks; 1.3 % PR rate
	Koh et al. (2013) [50]	49/everolimus	PR in 10.2 %, PFS 5.2 months
	Matrana et al. (2014) [64]	29/pazopanib	PFS/OS was 8.1/31.0 months for frontline use with 33 % RR; PFS/OS was 4.0/13.6 months in salvage setting with 6 %
	Voss et al. (2014) [124]	85/everolimus or temsirolimus	RR 7 %; SD 49 %; PFS/OS was 2.9 and 8.7 months

(continued)

Table 21.2 (continued)

Type	Study	N/agent	Relevant outcomes
Papillary RCC	Ratain et al. (2006) [88]	15/sorafenib	13 % PR
	Ronnen et al. (2006) [93]	3/sunitinib	33 % PR; PFS 8.5 months
	Choueiri et al. (2008) [15]	41/sorafenib or sunitinib	4.8 % PR; PFS of 7.6 months overall and 11.9 months with sunitinib
	Srinivasan et al. (2008) [103]	25/GSK1363089	16 % PR; 80 % SD
	Gordon et al. (2009) [38]	45/erlotinib	11 % PR; OS 27 months
	Ravaud et al. (2009) [89]	28/sunitinib	4 % PR; 57 % SD
	Stadler et al. (2010) [105]	107/sorafenib	3 % PR; SD 81 %
	Beck et al. (2011) [9]	112/sorafenib	~4 % PR;~6.6 month PFS
	Lee et al. (2012) [56]	31/sunitinib	22 patients with PRCC with ORR of 36 %; overall study PFS 6.4 months
	Tannir et al. (2012) [114]	55/sunitinib	27 patients with PRCC, PFS 1.6 months, OS 12.6 months; 0 % ORR
	Choueiri et al. (2013) [16]	Foretinib	13.5 % RR; PFS 9.3 months
	Escudier et al. (2013) [32]	92/everolimus	PFS 3.7 months; OS 21 months
	Stamatakis et al. (2013) [109]	34/erlotinib with bevacizumab	In sporadic cases, 25 % PR; 42.9 % PR in HLRCC
	Tannir et al. (2014) [113]	68/Sutent vs. everolimus with a crossover upon progression	27 patients with PRCC, OS 14.9 months for everolimus, 16.6 for sunitinib, 0 % ORR
	Yildiz et al. (2014) [130]	63/sunitinib	46 patients with PRCC; overall RR 11.1 %, PFS 7.6 months
Chromophobe RCC	Choueiri et al. (2008) [15]	12/sorafenib or sunitinib	25 % PR; 75 % SD; PFS 10.6 months
	Stadler et al. (2010) [105]	20/sorafenib	5 % PR
Collecting duct RCC (Bellini Duct)	Tannir et al. (2011) [112]	20/sunitinib or bevacizumab or other	Median OS of 421 days; one PR
	Pecuchet et al. (2013) [81]	Bevacizumab/ gemcitabine/ platinum	3 PRs, 1 SD, and 1 CR after a metastasectomy; mPFS 15.1 months and mOS 27.8 months
Xp11 translocation carcinoma	Malouf et al. (2010) [61]	11/sunitinib; 1/ temsirolimus	PFS 8.2 months; 9 % CR, 27 % PR; 55 % SD all with sunitinib
RCC with sarcomatoid dedifferentiation	Golshayan et al. (2009) [37]	43/sunitinib or sorafenib or bevacizumab	19 % PR; 49 % SD; PFS 5.3 months; OS 11.8 months
	Beck et al. (2011) [9]	53/sorafenib	PFS of 4 months

activating MET mutations [96, 100], histologically this tumor appears to be most similar to sporadic type 1 PRCC, and only about 14 % of patients with sporadic PRCC harbor a MET mutation [60, 97]. PRCC can also be seen in the hereditary leiomyomatosis and RCC syndrome due to fumarate hydratase (FH) tumor suppressor inactivation, and this appears to be histologically most similar to type 1 PRCC; FH mutations have not been described in sporadic cases [48, 55, 119]. Finally, vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFR) have been shown to also be expressed in PRCC, but the clinical correlation remains unclear [28, 58]. Tubulocystic renal cell carcinoma and mucinous, tubular, and spindle cell carcinoma are two newer renal cancer subtypes that have been proposed to be additional variants of PRCC [59, 102].

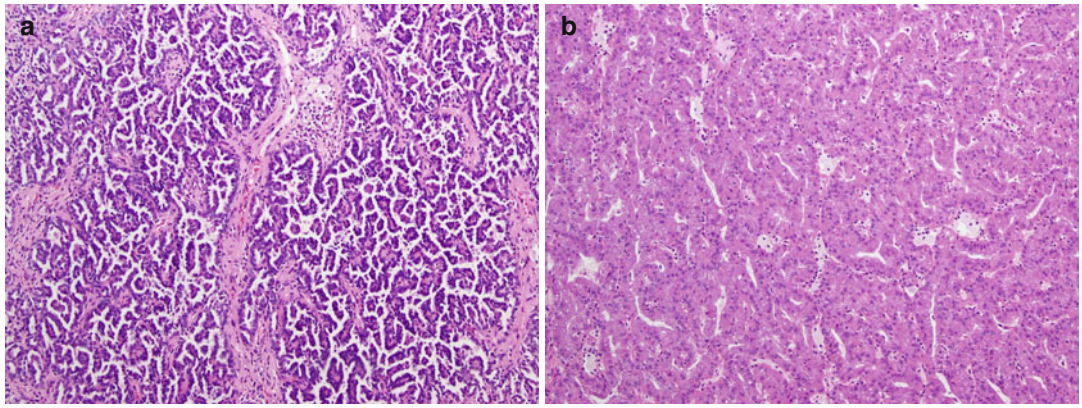

Fig. 21.1 (**a**) Papillary renal cell carcinoma type 1. The papillary cores with foamy macrophages are lined by small cuboidal cells with low-grade nuclei and minimal amount of cytoplasm. (**b**) Papillary renal cell carcinoma type 2. In this type of tumor, the papillary cores are lined by cells with abundant acidophilic cytoplasm and typically have high-grade nuclei with prominent nucleoli (Courtesy of Dr. Tatjana Antic, University of Chicago, Department of Pathology)

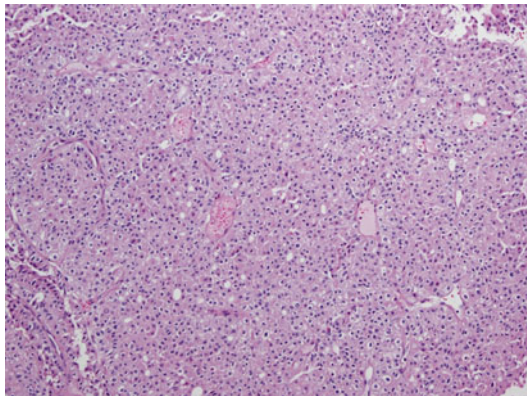

Fig. 21.2 Chromophobe renal cell carcinoma. The tumor cells are arranged in nests divided by interspersed thin-walled blood vessels. The cells contain eosinophilic cytoplasm with prominent cell membranes and dark resinoid nuclei with perinuclear halos (Courtesy of Dr. Tatjana Antic, University of Chicago, Department of Pathology)

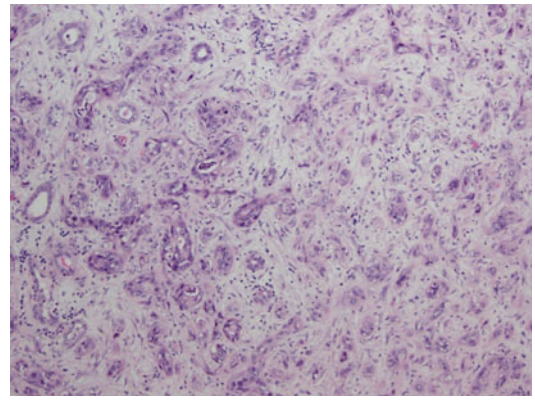

Fig. 21.3 Collecting duct carcinoma. Malignant cells with high-grade nuclear features arranged in tubules and cords are infiltrating the renal medulla. Pronounced desmoplastic stromal reaction is present (Courtesy of Dr. Tatjana Antic, University of Chicago, Department of Pathology)

21.2.2 Chromophobe RCC (CHRCC)

Chromophobe RCC (CHRCC) is thought to originate from the intercalated cells in the renal collecting ducts and accounts for approximately 4–10 % of RCC (Fig. 21.2) [4, 9, 19]. In familial chromophobe RCC associated with Birt-Hogg-Dubé (BHD) syndrome, inactivation of a tumor suppressor, folliculin, has been identified and may activate the mTOR pathway, but folliculin alterations have rarely been found in sporadic CHRCC [43, 75, 123]. VEGF and upregulation of c-KIT have been noted in tumor specimens, although activating mutations of c-KIT have not been found [28, 95, 110, 127].

21.2.3 Collecting Duct RCC (CDRCC)

Collecting duct RCC (CDRCC) likely arises from the collecting (Bellini) ducts of the kidney in the renal medulla and is an aggressive RCC subtype with approximately one third of patients presenting with metastatic disease (Fig. 21.3)

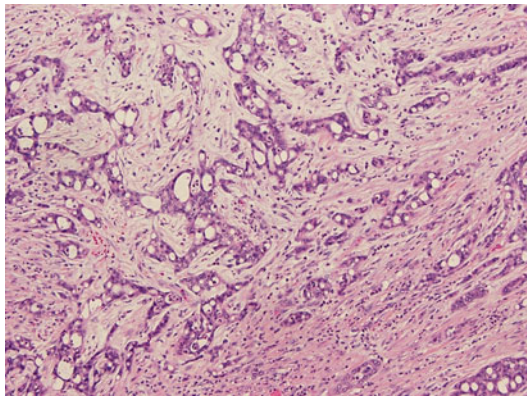

Fig. 21.4 Medullary renal cell carcinoma. The tumor is composed of fusing tubules and cords made of pleomorphic malignant cells in desmoplastic stroma. The acute inflammatory infiltrate is commonly seen in this type of tumor (Courtesy of Dr. Tatjana Antic, University of Chicago, Department of Pathology)

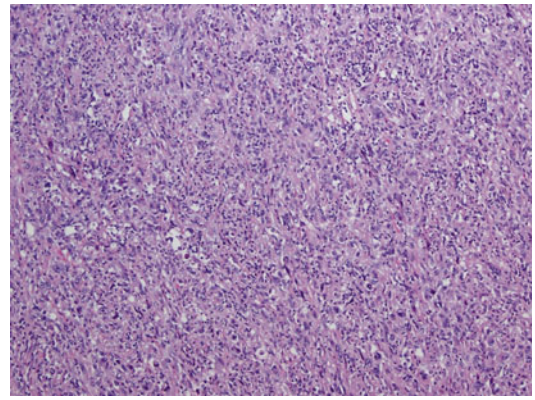

Fig. 21.5 Sarcomatoid dedifferentiation. The sarcomatoid change can be seen in any type of renal cell carcinoma with tumor showing highly pleomorphic cells in storiform pattern, numerous mitotic figures, and apoptotic bodies, simulating sarcoma-like appearance (Courtesy of Dr. Tatjana Antic, University of Chicago, Department of Pathology)

[69]. A relationship to urothelial cell carcinoma has been proposed [125].

21.2.4 Renal Medullary Carcinoma (RMC)

Renal medullary carcinoma (RMC), a rare, aggressive, and usually fatal RCC variant, is a close relative of CDRCC (Fig. 21.4) [22, 128]. Almost all RMC occurs in children and young adults with sickle cell trait or disease.

21.2.5 MiT Family Translocation RCC

MiT family translocation RCC involves different translocations of the TFE3, TFEB, TFEC, and MiTF transcription factors typically involving chromosome Xp11.2 but also including (6:11) translocations [6, 10, 102]. This was previously thought to be an extremely rare entity seen exclusively in children and young adults, but large series showed that 15 % of RCC patients under the age of 45 had this subtype of RCC. Although certain specific translocations can have indolent behavior, the majority of cases seen in adults are very aggressive [51].

21.2.6 Sarcomatoid Dedifferentiation

Sarcomatoid dedifferentiation, first described by Fallow et al. in 1968, is not a separate histologic subtype but rather a variant that is observed with any RCC histology, can involve 1–100 % of a given tumor, and is seen in 5–10 % of RCC based on a number of large surgical series; although, it has been described to occur up to 30 % in CDRCC [12, 23, 35]. It exhibits a spindle cell pattern of growth, is always a high-grade tumor, and has been associated with the expression of VEGF, c-Kit, PDGFR-alpha, and S6 kinase as well as p53 mutations and is associated with poor prognosis (Fig. 21.5) [24, 78, 83, 117].

21.3 Survival Considerations: Non-clear Cell Renal Cell Carcinoma

Because optimal therapies for NCCRCC remain unknown, available therapies can have significant toxicities, and certain subtypes have an indolent natural history; knowledge of expected survival in the absence of treatment is critical to

therapeutic decision-making. Most studies evaluated outcome in surgical series of primary nephrectomies, but with those caveats, we will review the salient findings.

Localized PRCC and CHRCC have in some studies, but not others, been shown to have an improved survival compared to localized CCRCC. In a large multicenter retrospective series of 4,063 patients for those with localized disease, 5-year survival rates were 73.2 %, 79.4 %, and 87.9 % for clear cell, papillary, and chromophobe carcinoma, respectively, but once adjusted for TNM stage, no significant survival difference was observed [80]. A study of 2,385 patients treated at the Mayo Clinic from 1970 to 2000 found the 5-year cancer-specific survival for the entire group to be 68.9 % in those with CCRCC, 87.4 % in PRCC, and 86.7 % in CHRCC patients with CCRCC patients having a statistically worse outcome even after stratifying for tumor stage and nuclear grade ($P<0.001$) [13]. CHRCC has a low rate of metastasis (~5 %) with a 5-year OS of 92 % seen in a series of 50 patients and has been shown to have a statistically significant less chance of disease recurrence compared to CCRCC after a nephrectomy [4, 8, 19, 116].

Metastatic PRCC and CHRCC appear in most studies to have a worse prognosis compared to CCRCC. A study of 64 patients with metastatic non-clear cell RCC treated with both cytokine and conventional chemotherapy agents found that only two had a partial response with a median OS of 9.4 months with 29 months for those with CHRCC, 11 months for those with CDRCC, and 5.5 months for those with PRCC [73]. These same investigators reviewed 353 previously untreated metastatic RCC, of whom 13 % had NCCRCC (mostly papillary), and those with CCRCC had a significantly better survival than those of NCCRCC [66]. Another series of 38 patients with metastatic PRCC had an OS of 8 months, and a separate single-center study found a significant difference in survival of patients with metastatic RCC after a cytoreductive nephrectomy with 9.1-month median OS in those with PRCC vs. 22 months for CCRCC [63, 93]. A retrospective analysis of the International mRCC Database Consortium (IMDC) included 252 patients with NCCRCC of whom 151 had PRCC and 37 had CHRCC [53]. The median OS for the NCCRCC subgroup was 12.8 months compared to 22.3 months in the clear-cell RCC group. Interestingly, the median OS for PRCC was 14.0 months (adjusted hazard ratio for death 1.57 compared to CCRCC, $p<0.0001$) versus 27.1 months for those with CHRCC (adjusted hazard ratio for death 0.89 compared to CCRCC, $p=0.646$).

CDRCC and RMC patients have in general uniformly poor survival even when localized. A study of 160 cases noted the 3-year disease-specific survival was 58 % compared to 79 % for CCRCC and for those with metastatic disease a median OS of 5 months for CDRCC versus 8 months in those with CCRCC [125]. In another study of 26 patients with metastatic CDRCC, the median OS was 11 months with a 5 % 2-year survival [73]. CDRCC OS at 5 and 10 years for a cohort of 81 patients in Japan was 34.3 % and 13.7 %, respectively, with 32 % presenting with metastatic disease [118]. For RMC, mean survival in several series has been approximately 4 months [111].

Sarcomatoid dedifferentiation has clearly been demonstrated to be a poor prognostic marker. In a large series of 2,381 patients, 120 (5 %) of whom had a sarcomatoid component in various stages of RCC, the 5-year cancer-specific survival was 14.5 %, and the presence of a sarcomatoid component was significantly associated with death [12]. Sarcomatoid dedifferentiation has also been shown to be an independent poor prognostic marker in metastatic RCC in those treated with cytokine therapy with one study showing a median OS to be 22 months vs. 10 months in those treated with cytokines and having no sarcomatoid vs. sarcomatoid features [54, 57, 62].

In summary, when localized, CCRCC appears to have a worse prognosis than PRCC or CHRCC, but this is not necessarily true in the metastatic setting, including under treatment with cytokines or conventional chemotherapy, which have minimal to no efficacy. CDRCC/RMC and

sarcomatoid dedifferentiation lead to abysmal outcomes. Outcomes with conventional chemotherapy, cytokines, VEGF pathway, and mTOR-targeted therapies for NCCRCC are discussed below.

21.4 DNA and DNA Repair-Targeted Therapy of Non-clear Cell Carcinoma

Conventional cytotoxic chemotherapy has not been considered to be useful in the treatment of RCC; nevertheless, objective responses have been reported using nucleoside analog-based therapies with the highest response rates reported utilizing a combination of gemcitabine and a fluoropyrimidine (capecitabine most often) or gemcitabine with doxorubicin [18, 68, 104, 106, 115, 126]. Application of these and other cytotoxic therapies in the NCCRCC specifically is summarized below [21, 44].

21.4.1 PRCC

PRCC is in general resistant to conventional chemotherapy [17]. A retrospective study of 38 patients with metastatic PRCC, 30 of whom were treated with systemic therapy of which six received conventional chemotherapy, showed no objective responses [93]. In a study of 153 patients treated with gemcitabine and 5-fluorouracil, two had definite PRCC and neither showed an objective response [107]. A phase I study of gemcitabine, capecitabine, IFN-α, and thalidomide in 12 patients included two with PRCC, one of whom had a partial response [3]. An analysis of 18 patients with PRCC treated with various agents including conventional chemotherapy showed no significant responses [73]. A phase II study of 45 patients with mRCC receiving S-1 (an oral formulation combining tegafur, 5-chloro-2,4-dihydropxypyridine, and potassium oxonate) included eight patients with PRCC; ORR was 12.5 % and PFS 5.9 months [76]. Another phase II study of carboplatin and paclitaxel for 16 PRCC patients showed no responses [11]. Finally, a phase II study of single-agent capecitabine in previously untreated metastatic NCCRCC patients enrolled 51 individuals (39 PRCC, 7 CHRCC, 5 CDRCC) most of whom had an intermediate MSKCC risk score and all of whom had a prior nephrectomy and surprisingly high response rates of 2 complete responses (CR), 11 partial responses (PR), and 24 with stable disease (SD) with a median PFS, and OS of 10.1 months and 18.3 months, respectively, was reported [120]. One of the CRs occurred in a PRCC patient.

21.4.2 CHRCC

CHRCC has rarely been evaluated in regard to conventional chemotherapy use, but in 12 individuals in a series of 64 patients with metastatic NCCRCC, none had a response to conventional chemotherapy, although specific agents used were not specified [73]. One CR out of seven CHRCC patients was observed in a series of 51 NCRCC patients treated with single-agent capecitabine [120]. No responses were seen in two patients with CHRCC receiving pemetrexed plus gemcitabine [91].

21.4.3 CDRCC and RMC

CDRCC and RMC treatment with conventional chemotherapy has been evaluated in a number of retrospective series and case reports due to histologic similarities between CDRCC and urothelial carcinoma. In a series of 64 patients with NCCRCC, 26 had collecting duct/medullary histology, and one had a 5-month PR to gemcitabine plus cisplatin therapy [73]. A series of 12 patients with CDRCC treated most commonly with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) reported only one response lasting 5 months [27]. The largest retrospective series of CDRCC included 81 patients from Japan and included 26 patients with distant metastatic disease. Almost everyone was initially treated surgically, and 17 individuals were treated postoperatively with chemotherapy with only a

single response to combination of gemcitabine and carboplatin [118]. In a separate study of nine patients with CDRCC treated with gemcitabine and cisplatin, two CRs were noted [84]. This was followed by a prospective phase II trial of gemcitabine and cisplatin or carboplatin for 23 treatment-naive metastatic CDRCC patients from six French centers with an objective response (CR + PR by Response Evaluation Criteria in Solid Tumors) rate of 26 % (one CR and five PR) and a median PFS and OS of 7.1 months and 10.5 months, respectively [79]. The previously mentioned small phase II trial of pemetrexed and gemcitabine had no activity in three CDRCC patients and in NCCRCC in general [91]. Finally, a series of five patients treated with gemcitabine, platinum, and bevacizumab with bevacizumab maintenance led to three cases of PR, one case of SD, and one CR after a metastasectomy; mPFS was 15.1 months and mOS 27.8 months [81].

21.4.4 Sarcomatoid Dedifferentiation

Sarcomatoid dedifferentiation leads to an aggressive growth pattern and uniformly poor prognosis. Early studies of MAID (mesna, adriamycin, ifosfamide, dacarbazine), gemcitabine/docetaxel/carboplatin, and doxorubicin-based CYVADIC treatments showed limited success [7, 20, 45, 98]. A phase II trial of doxorubicin and ifosfamide in 25 patients with metastatic sarcomatoid RCC showed no objective response and a median OS of 3.9 months, although case reports of CRs to the same chemotherapy can be found [33, 87]. Eighteen patients with sarcomatoid features treated with a combination of doxorubicin and gemcitabine had two CRs, five PRs, and one SD (ORR = 39 %), and long-term follow-up of four patients found the two complete responders alive 6 and 8 years later and the other two surviving over 3 and 6 years [30, 77]. An ECOG phase II trial of doxorubicin and gemcitabine in 39 patients with sarcomatoid features showed a 16 % response rate (five PRs and one CR) and 10 (26 %) with SD with a median OS of 8.8 months and a median PFS of 3.5 months [41]. It also appeared that those tumors with a higher sarcomatoid component responded better to the chemotherapy. Of note, these trials made no attempt to distinguish the histologic origin or specific renal cancer subtype. A trial of sunitinib with or without gemcitabine for mRCC patients with sarcomatoid features is ongoing (clinicaltrials.gov NCT01164228).

In summary, nucleoside analog therapy appears to have similar low level activity in PRCC and CHRCC as it does in CCRCC, with the caveat that the clinical significance is unknown. CDRCC occasionally responds to agents typically utilized for urothelial cancer, but complete responses are extremely rare, and response rates and response durations appear to be less than in typical urothelial cancer. Reports of complete responses with gemcitabine and doxorubicin in RCC with sarcomatoid differentiation remain intriguing but have not been uniformly replicated, and it has not been possible to determine whether such responses are limited to any specific RCC subtype.

21.5 Cytokine Therapy of Non-clear Cell Renal Cell Carcinoma

Although cytokine therapy has been useful in CCRCC with responses seen in up to 20 % of patients with complete responses in 5–10 % of patients treated with high-dose IL-2, it has not been successful in NCCRCC [18]. In an analysis of 163 cases of metastatic RCC treated with IL-2 for whom kidney specimens were available and who were treated between 1990 and 2001, 146 were CCRCC and 17 NCCRCC with two PRCC type 1, nine PRCC type 2, two chromophobe, and one CDRCC. Response rate (>50 % regression of measurable tumor) to IL-2 was observed in 30 of the CCRCC patients (21 %) versus one of the NCCRCC patients (6 %) which was reportedly a CDRCC case [121]. Preliminary results of a prospective single-arm SELECT trial, with 120 patients treated with high-dose IL-2, showed an objective response in 0 out of five NCCRCC patients [65]. A review of 31 patients with

NCCRCC treated with either IL-2, IFN-α, or the combination of the two revealed one PR in a patient with CHRCC treated with IFN-α [73]. Dimopoulos et al. treated six CDRCC patients with a combination of IL-2 and IFN-α with one PR and two SDs [27]. Tokuda et al. found no response to immunotherapy in 34 patients treated with CDRCC [118]. Finally, a study of 108 patients with sarcomatoid features, but no information on the underlying histology, of whom 80 received some form of immunotherapy either alone or in combination with conventional chemotherapy, revealed 28 PRs and no CRs, with an OS for the entire cohort of 9 months [67]. Thus, although cytokine therapy is useful in select cases of metastatic CCRCC with CR being occasionally observed, there is little evidence to support its use in those with NCCRCC.

21.6 VEGF Pathway-Targeted Therapy of Non-clear Cell Renal Cell Carcinoma

The VHL/HIF pathway that is abnormal in most patients with sporadic CCRCC is not thought to be a major driver in any NCCRCC; nevertheless, VEGF and VEGF receptors are present and apparently overexpressed, at least in PRCC and CHRCC [58]. Multiple VEGF pathway-directed agents have been approved for CCRCC based on a number of large phase III trials which showed improved PFS and trends toward OS benefit in the metastatic CCRCC setting [31, 34, 74, 92]. Not surprisingly, these agents have also been investigated in NCCRCC; although, there are limited prospective evaluations.

21.6.1 Papillary RCC

In the randomized discontinuation trial of sorafenib, 15 patients with PRCC were included and two attained a PR [88]. A retrospective analysis examining 41 metastatic PRCC patients treated with sorafenib or sunitinib between 2002 and 2006 at four European and one American center showed a PFS of 7.6 months, with a response rate of 4.8 % (two patients, both treated with sunitinib and achieving a PR) and a PFS of 11.9 vs. 5.1 months in those treated with sunitinib vs. sorafenib ($P<0.001$) [15]. Two studies, the EU-ARCCS (Advanced Renal Cell Carcinoma Sorafenib) and the North American ARCCS, examined sorafenib use in a community-wide, expanded-access manner and analyzed its data on NCCRCC [8, 101]. The North American ARCCS trial evaluated 1,891 patients for RECIST response of whom 107 had PRCC. The CR, PR, SD, and CR+PR+SD rates for all patients and PRCC were CR=<1 %, 0 %; PR=4 %, 3 %; SD=80 %, 81; CR+PR+SD=84 %, 84 %, respectively. The EU-ARCCS looked at 1,150 patients, with a 4 % PR rate and a PFS of 6.6 months; a subset analysis of those with papillary features ($n=112$) found the PR and PFS to be similar to the clear-cell subset [8]. An expanded-access trial of sunitinib of over 4,300 patients, 68 % of whom had prior cytokine therapy and 3,464 of whom were evaluable, showed an overall objective response (CR +PR) of 17 % (1 % CR) and SD of >3 months in 59 % [39]. In the study, there were 437 (13 %) NCCRCC (not further subclassified) patients able to be evaluated, and an ORR of 11 % (2 CRs) and SD of 57 % was achieved, both comparable to the entire cohort. A phase II sunitinib trial enrolled 25 PRCC patients of whom 74 % had either poor or intermediate risk by MSKCC criteria and no PRs or CRs were seen, and median PFS and OS were only 1.6 and 12.6 months, respectively [110]. Another phase II trial of sunitinib in patients with NCCRCC included 22 evaluable patients, eight of whom had PRCC and achieved a PFS of 5.6 months with OS not reported [70]. A phase II trial of sunitinib in five patients with type 1 PRCC and 23 patients with type 2 PRCC showed a PR in one type 2 patient with 13 SD and 3/5 patients with SD in the type 2 [86]. A multicenter phase II trial from South Korea reported on 31 NCCRCC patients, of whom 22 had PRCC, treated with sunitinib; for the entire cohort, the median PFS was 6.4 months, and for the PRCC patients, the ORR was 36 % [55]. Another multicenter trial from the Turkish Oncology Group reported on 63 NCCRCC patients treated with sunitinib with 88 % having PRCC [126]. Overall study RR and disease

control rate was 11.1 % and 63.5 %; median PFS and OS were 7.6 months and 22 months. Finally, two prospective trials of sunitinib versus everolimus in NCCRCC have been completed, and one has been reported in preliminary fashion [109]. This trial was aborted early due to improved overall survival in the sunitinib arm. Notably, 27 of 68 enrolled patients had PRCC, and their estimated median OS in the sunitinib and everolimus arms was 16.6 and 14.9 months, respectively; notably, no objective responses with first-line therapy in PRCC patients were seen. Differences in outcomes seen between the expanded-access trials and the phase II data may be related to lack of central pathologic review in the large trials as well as a selection bias and possibly ethnic differences in response to the phase II trials.

21.6.2 Chromophobe RCC

A retrospective analysis of 12 patients with metastatic CHRCC treated with sorafenib or sunitinib found the PFS to be 10.6 months, with a response rate of 25 % (two patients treated with sorafenib and one with sunitinib) and SD in the remaining nine patients of more than 3 months [15]. Two of five patients with chromophobe RCC responded in a prospective study of sunitinib in NCCRCC, two of six patients with chromophobe RCC responded in the sunitinib versus everolimus trial, and one out three patients responded in the prospective sunitinib in NCCRCC trial [55, 109, 110]. In the North American ARCCS trial, 20 patients with CHRCC were evaluated and found to have CR and PR of 0 % and 5 %, respectively, all similar to the overall group and the PRCC subset [101].

21.6.3 Collecting Duct and Medullary

A case report of a 55-year-old woman with metastatic CDRCC who was treated with neoadjuvant sorafenib 400 mg twice daily and achieved both a 30 % reduction of primary tumor size and regression of nodal metastasis followed by a cytoreductive nephrectomy and continual sorafenib has been reported [5]. Several other case reports using sunitinib showed a mixed response with one achieving a PR of 7 months and another showing two responses [70, 108]. A case series of seven patients from a single center with CDRCC, five of whom were treated with VEGF-directed therapy (sorafenib in four patients and sunitinib in one patient), showed a single long-lasting response with one patient treated with sorafenib followed by sunitinib living 49 months [86]. A retrospective review from four US centers of 20 RMC patients treated between 2000 and 2010 revealed that 19 presented with stage III or IV disease, and of the 16 patients able to be evaluated, median OS at 722 days was 421 days with 13 patients dead [112]. Treatment in the preceding study was with various agents, including sunitinib in five patients and bevacizumab plus other agents in three patients. Of note, of the 15 patients who had any targeted therapy at any point, only one PR was noted. Finally, none of six patients with CDRCC responded to sunitinib in a prospective NCCRCC trial [114].

21.6.4 Sarcomatoid Dedifferentiation with Any Histologic Type

The EU-ARCCS trial included 53 patients with sarcomatoid dedifferentiation and found the PFS to be approximately 4 months, significantly less than the PFS for the entire cohort of 6.6 months [9]. In a retrospective series of 43 patients (33 of whom had CCRCC as the underlying primary histology) with sarcomatoid features treated with sunitinib, sorafenib, or bevacizumab, a PR was observed in 19 % and SD in 49 % with the PRs limited to those with less than 20 % sarcomatoid features [37]. Median PFS and OS were 5.3 months and 11.8 months. Of note, all PRs were seen in those with underlying CCRCC, and in that group of 33, the PFS and OS were 6 and 13.1 months. When matched with a group of CCRCC patients without sarcomatoid features, the PFS was 6.2 vs. 16.3 months in those with and without sarcomatoid features ($P<0.001$). These results appear to be more promising than those achieved with conventional chemotherapy or cytokine therapy in the past.

21.6.5 Other Subtypes

Several reports of MiT translocation RCC treated with targeted agents have been published. The largest series to date includes 23 patients with metastatic Xp11.2 translocation RCC of which 11 received sunitinib and one temsirolimus as first-line therapy [60]. The median PFS was 8.2 months in the sunitinib group versus 2 months in the cytokine group (nine patients) with one CR, three PRs, and six SDs in the sunitinib group, and at the time of analysis, OS was not reached in the sunitinib group vs. 17 months in the cytokine group. Many of the patients who failed sunitinib were able to go on to sorafenib, temsirolimus, or everolimus treatment. A separate American retrospective series revealed that 3 of 15 patients responded to a VEGF pathway-targeted therapy, and the PFS of the entire cohort was 7.1 months [14].

In summary, VEGF pathway inhibitors appear to have a real effect on NCCRCC. In PRCC, PRs are generally in the 0–10 % range with very rare CRs, but SD was noted to be anywhere from 60 % to 80 %, although data from the expanded-access trials was much more optimistic than the smaller phase II data. In CHRCC, a response rate of up to 25 % was observed, and even in those with sarcomatoid dedifferentiation, a PR and SD of 19 % and 49 % were seen. Finally, response rates of approximately 20 % are also seen in patients with Xp11.2 translocation cancers. Taken together, these data support the use of agents targeting the VEGF pathway as first-line treatment over conventional chemotherapy or immunotherapy, except perhaps in the case of CDRCC where conventional chemotherapy may still have a role.

21.7 mTOR Pathway-Targeted Therapy of Non-clear Cell Renal Cell Carcinoma

The mammalian target of rapamycin (mTOR) is a protein kinase in the PI3K-Akt pathway involved in cellular growth and proliferation and response to hypoxia [47]. The activation of this pathway leads to increase in HIF and angiogenesis. Furthermore, PTEN (phosphatase and tensin homologue deleted on chromosome 10) which negatively regulates Akt activation has been shown to be decreased in RCC, thus leading to increase in Akt activity and providing more support for targeting of this pathway [42]. Thus, temsirolimus and everolimus, both inhibitors of mTOR, have been developed as targeted therapy for metastatic RCC.

21.7.1 Papillary RCC

By far, the largest trial examining mTOR inhibitors in NCCRCC was done as part of the phase III ARCC trial looking at temsirolimus, IFN-α, or both for advanced RCC [46]. The trial of 626 patients, which showed an OS advantage to temsirolimus (10.3 months) versus IFN-α (7.3 months) or both (8.4 months), enrolled 20 % NCCRCC and required no previous systemic therapy as well as at least three adverse prognostic markers. Exploratory subgroup analysis of NCCRCC patients showed equivalent median OS for those with CCRCC (10.7 months) vs. NCCRCC (11.6 months) if treated with temsirolimus but worse median OS for those with NCCRCC (4.3 months) vs. CCRCC (8.2 months) if treated with INF-α [29]. When examining IFN-α vs. temsirolimus in the NCCRCC subgroup, the hazard ratio for death for treatment with temsirolimus was 0.49 (95 % CI = 0.29, 0.85), and with the caveat of no central pathologic review, PRCC histology was noted in over 75 % of the NCCRCC cases with CHRCC in somewhere between 10 % and 15 %. A retrospective review of mTOR-directed therapy in NCCRCC included 14 patients with PRCC [124]. Although overall PFS and OS for the entire cohort of NCCRCC was a short 2.9 and 8.7 months (>60 % of the cohort had prior therapy), three PRCC patients were treated for >1 year. Preliminary data from a prospective study of everolimus in PRCC in 92 patients revealed that the overall PFS was 3.7 months [32]. In a similar Korean study, 2/28 PRCC patients responded to everolimus [50]. The preliminary data for a small prospective trial of sunitinib versus everolimus are noted above.

21.7.2 Chromophobe RCC

Data from the IFN-α versus temsirolimus prospective study are noted above [29]. In the aforementioned retrospective review, two of nine CRCC were treated with an mTOR inhibitor for >1 year [124]. Similarly, 2/7 CRCC responded to everolimus in the Korean prospective study [50]. In the preliminary report of everolimus versus sunitinib in NCCRCC, 12 patients with CRCC were enrolled, and there was a suggestion of improved outcome in the sunitinib cohort [113].

There is minimal data in response to mTOR inhibitors in the other tumor subtypes, mainly due to their underrepresentation in both retrospective and prospective studies in NCCRCC. Notably, molecular predicators of benefit to mTOR inhibitors have been described and are being evaluated in renal cancer patients as well [49].

21.8 Targeted Therapy of Non-clear Cell Renal Cell Carcinoma: Novel Pathways

21.8.1 EGFR Pathway

Based on preclinical work showing that wild-type VHL gene expression is necessary for effective anti-EGFR therapy and knowing that most PRCC harbor a wild-type VHL gene, a phase II trial of erlotinib, an EGFR TKI, was conducted [38, 82]. The trial enrolled 45 evaluable PRCC patients with an overall response rate of 11 % (all 5 PRs) and a median OS of 27 months, 6-month probability of freedom from treatment failure of 29 %, with one death due to pneumonitis. Interestingly, no correlation between EGFR expression and response to therapy was noted. A study of 88 patients of whom 14 had NCCRCC and who were treated with panitumumab, a chimeric monoclonal antibody against the EGF receptor, showed two PRs and six SDs and a median PFS of 92 days in the NCCRCC patients (exact subtype not specified) [94]. Preliminary results from an ongoing study of erlotinib with bevacizumab in patient with hereditary leiomyomatosis and renal cell cancer (HLRCC) as well as sporadic PRCC were reported at the 2013 European Cancer Organization (ECCO) meetings [109]. To date, 34 patients have been enrolled, including 20 in the sporadic cohort. The ORR was 32.4 % with a DCR of 64.7 % at 24 weeks. Importantly, 5/20 sporadic PRCC patients had a PR as well as 6/14 with HLRCC. Median PFS for the sporadic cohort was 7.3 months with a median follow-up of 10.7 months.

21.8.2 MET Pathway

A molecular study of 220 localized PRCC demonstrated genomic copy number gains in 46 % of type II PRCC and 81 % of type 1 PRCC [1]. MET gene mutations were identified in 22 % of type 1 PRCC. A case report of response to an investigational MET inhibitor in a PRCC patient with tumor MET mutation has been published [26]. The best data for MET pathway targeting comes from a phase II study of the MET/VEGFR inhibitor foretinib in papillary renal cancer [16]. In this study in which 74 patients were enrolled, 11 patients had germline MET mutations and five additional patients had tumor-specific MET mutations. Eighteen and two of 42 patients with adequate tissue had chromosome 7 or MET amplification, respectively. In the entire trial, overall response rate was 13.5 %, and median PFS was 9.3 months. Five of ten patients with germ line MET mutations responded, but other MEt alterations did not appear to correlate with response. It is not clear whether the inhibition of MET, VEGFR2, or the combination of the two led to the above results. Nevertheless, prospective evaluations of more potent and specific MET inhibitors in PRCC are underway.

21.9 Immunotherapy

Cytokine therapy, specifically with IL2 and/or IFNA, is discussed in section 17.5 above. A separate chapter on checkpoint inhibitors, specifically PD1 and CTLA4 pathway inhibitors, discusses this emerging therapy and its role in RCC therapy

in detail. All the studies to date have focused on CCRCC, and there is limited to no data available in the NCCRCC.

Conclusions

Treatment of advanced NCCRCC remains challenging due to the generally aggressive nature of the disease and a lack of good therapeutic options. A recent meta-analysis of systemic therapy for NCCRCC found an overal response rate of 10.5 % and a median PFS and OS of 7.4 and 13.4 months for those treated with targeted therapy, significantly lower compared to CCRCC [118]. A paucity of randomized prospective trials for most of the subtypes makes treatment decisions difficult, and thus, future discovery of novel pathways involved in NCCRCC and rational design of drugs to target those pathways as well as clinical trials specifically tailored to NCCRCC are vital. VEGF and mTOR pathway inhibitors have shown some activity in NCCRCC and should be considered as first-line therapy for the majority of patients, with the best data supporting use of VEGF pathway inhibitors first. Nevertheless, overall response and benefit are limited, and more specific therapy based on identified molecular pathways and participation in clinical trials is preferable.

Clinical Vignette

A 65-year-old-man presented to his primary care physician with 2 months of intermittent hematuria. A CT scan of the abdomen and pelvis revealed a 4.7 cm mass in the upper pole of the right kidney with no evidence of distant disease. The patient underwent a nephrectomy with pathology showing a Fuhrman grade 3 PRCC type 2 with negative margins. No adjuvant therapy was given, and 6 months later, the patient experienced disease recurrence in the retroperitoneum and lungs not deemed to be resectable.

Since no clinical trial was available, the patient was initiated on sunitinib at 50 mg daily on a 4 weeks on, 2 weeks off schedule. He tolerated the drug well, with minimal diarrhea and skin changes, and proceeded to have stable disease (SD) for the following 9 months. Following PD in the lungs, the patient was switched to temsirolimus at 25 mg per week and once again had SD for 7 months at which point PD occurred once again in the lungs and now the liver.

Currently, the patient has been switched to sorafenib at 400 mg twice a day for the last 3 months with SD once again. This case underscores the current paradigm for treatment of metastatic RCC, which is to use sequential targeted therapy in the hopes of establishing long-term disease stability with minimal side effects of treatment, converting the disease into a chronic illness.

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Toxicity Management of Renal Cell Cancer Patients on Targeted Therapies

22

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Contents

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22.1 Introduction

Targeted therapies have significantly changed the treatment landscape for patients with metastatic renal cell carcinoma (mRCC). TKIs such as sunitinib, sorafenib, pazopanib, and axitinib are all multi-targeted inhibitors which inhibit a variety of targets including the vascular endothelial growth factor receptors 1, 2, and 3, platelet-derived growth factor receptor (PDGFR), and others [1–5]. Temsirolimus and everolimus both interfere with angiogenesis by inhibiting mTOR, a critical regulator within the cell [6, 7]. Bevacizumab blocks the vascular endothelial growth factor (VEGF) pathway by binding to VEGF [8].

It is now widely accepted that these targeted agents have a unique mechanism of action and are associated with a distinct and unique pattern of toxicities. While targeted agents generally have an acceptable toxicity profile, some side effects require careful monitoring and treatment in order to achieve optimal patient outcomes. In clinical practice, the most common side effects of

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_22

targeted agents are fatigue/asthenia, anorexia/loss of appetite, hand-foot syndrome (HFS) stomatitis/taste changes, diarrhea/abdominal pain, myelosuppression, and hypertension, while mTOR inhibition frequently is associated with mucocutaneous side effects, metabolic disturbances such as hyperglycemia and hyperlipidemia, and pneumonitis.

Three key interlinked areas have emerged as being essential for the optimal use of targeted agents in mRCC: dosing and schedule, treatment duration, and proactive side effect management. Only if all of these three key areas are optimized will the maximum benefit be achieved for each patient. Unlike conventional chemotherapy, targeted agents are given continuously as long as the patient benefits, which in some cases may extend for several years. This continuous treatment application makes side effect management critical and requires individualized management of the delicate balance between toxicity and dose intensity in order to maximize quality of life as well as patient benefit.

Knowledge about and optimal proactive management of acute side effects is therefore essential and may help to reduce patient discomfort and avoid unnecessary dose reductions, treatment interruptions, or even early treatment discontinuation. Patients undergoing treatment with targeted agents should be monitored by a qualified physician and/or oncology nurse experienced in the use of anticancer agents and should be counseled on the potential for treatment-related side effects, including the importance of maintaining optimal dose and therapy duration.

22.2 Importance of Dosing and Schedule

A significant relationship between drug exposure and efficacy/toxicity has been identified for several agents including sunitinib, sorafenib, axitinib, pazopanib and bevacizumab [9–12]. Patients with the highest exposure to sunitinib not only displayed a higher probability of a response and tumor shrinkage but also longer time to progression and, most importantly, longer overall survival [9]. Sorafenib when dose intensified appeared to have a substantially higher response rate than at standard doses [10, 13]. Similarly, bevacizumab at 10 mg/kg body weight was more active than at 5 mg/kg body weight [11]. A greater proportion of axitinib patients achieved an objective response when dose was titrated as compared to standard-dose patients [12]. This underscores the great importance of dose titration and maintaining patients on the maximum dose tolerated and striving to avoid any unnecessary dose reductions during treatment. Furthermore, minimizing the time off therapy is important, since tumor progression may occur rapidly during treatment interruption. Patients should always be started on the recommended dose, while lower starting doses should only be considered if there are significant concerns about potential toxicity.

Toxicity appears also to correlate with drug exposure as, for example, shown for sunitinib-induced neutropenia and fatigue; axitinib-induced hypertension; temsirolimus-induced thrombocytopenia, hyperlipidemia, hyperglycemia, and mucocutaneous side effects; and pazopanib-induced diarrhea, hand-foot syndrome, or mucositis [9, 14, 15]. The observed interindividual variability in toxicity can be related to variability in oral absorption and drug clearance, ethnic differences, gender differences, and SNPs [16–20]. In case of significant uncontrollable toxicities, individualized dose reductions and schedule changes can be considered depending on the nature of the toxicity, its severity, and its timing in the treatment schedule. Such individualized schedule changes have been studied in small subsets of patients [21–24]. A 2-week-on/1-week-off schedule for sunitinib allows the delivery of the same dose intensity over a 6-week period as the 4-week-on/2-week-off schedule but appears to be better tolerated by the majority of patients in particular by patients with significant side effects in weeks 3 and 4 [24–26]. However, these schedules need to be confirmed in prospective studies and should currently not be used as standard schedules but be reserved for those patients who struggle with tolerability.

22.3 Toxicity and Toxicity Management

Tables 22.1 and 22.2 give an overview of selected toxicities and their frequencies of currently approved TKIs and mTOR inhibitors as observed in pivotal phase III studies. Most toxicity data and studies examining potential mechanism of different toxicities are available for sunitinib. Some side effects or their full extent only became evident during the pivotal phase III study, e.g., hypothyroidism and cardiotoxicity, and subsequent studies utilizing thorough screening confirmed higher frequencies than described in the phase III study.

22.4 Fatigue and Asthenia

Fatigue and asthenia represent some of the most frequently encountered targeted agent-related side effects [2, 6, 7, 27–29]. Fatigue may be

Table 22.1 Selected treatment-related toxicities of TKIs reported in phase III trials: sunitinib, sorafenib, pazopanib, and axitinib

	Sunitinib		Sorafenib		Pazopanib		Axitinib	
Toxicity	All grade %	Grade 3/4 %	All grade %	Grade 3/4 %	All grade %	Grade 3/4 %	All grade %	Grade 3/4 %
General toxicities								
Fatigue	31	5	33	3	14	3	39	11
Anorexia/asthenia	22	2	4	3	22	2	21	5
Infections	37	10	27	5	n/r	n/r	n/r	n/r
Gastrointestinal toxicities								
Nausea	15	0	37	2	26	<1	32	3
Vomiting	12	0	19	2	21	2	24	3
Diarrhea	17	1	27	1	62	3	55	11
Mucositis/stomatitis	14	1	20	1	<10	<1	15	1
Dermatologic toxicities								
Rash	25	<1	n/r	n/r	<10	<1	13	<1
Hand-foot syndrome	n/r	n/r	n/r	n/r	<10	<1	27	5
Cardiovascular/respiratory toxicities								
Hypertension	30	12	17	4	13	<1	40	16
LVEF decrease	13	3	n/r	n/r	n/r	n/r	n/r	n/r
Dyspnea	10	2	14	4	n/r	n/r	n/r	n/r
Pneumonitis	n/r	n/r	n/r	n/r	n/r	n/r	n/r	–
Hematologic toxicities								
Anemia	79	8	8	3	n/r	n/r	35	<1
Neutropenia	77	18	n/r	n/r	34	1	6	1
Thrombocytopenia	68	9	n/r	n/r	32	<1	15	<1
Laboratory/metabolic toxicities								
Hyperglycemia	n/r	n/r	n/r	n/r	41	<1	n/r	n/r
Hypercholesterolemia	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Hypertriglyceridemia	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
Hypophosphatemia	31	6	n/r	n/r	34	4	13	2
Hyperbilirubinemia	19	1	n/r	n/r	36	3	n/r	n/r
Hypercreatinemia	66	1	n/r	n/r	<10	<1	55	0
Increase in AST	52	2	n/r	n/r	53	7	n/r	n/r
Increase in ALT	46	3	n/r	n/r	53	12	n/r	n/r
Hypothyroidism	14	2	n/r	n/r	<10	<1	19	<1

n/r not reported

Table 22.2 Selected treatment-related toxicities of mTOR inhibitors reported in phase III trials: everolimus and temsirolimus

	Everolimus		Temsirolimus	
Toxicity	All grade %	Grade 3/4 %	All grade %	Grade 3/4 %
General toxicities				
Fatigue	31	5	33	3
Anorexia	22	2	4	3
Infections	37	10	27	5
Gastrointestinal toxicities				
Nausea	15	0	37	2
Vomiting	12	0	19	2
Diarrhea	17	1	27	1
Mucositis/stomatitis	14	1	20	1
Dermatologic toxicities				
Rash	25	<1	–	–
Hand-foot syndrome	–	–	–	–
Cardiovascular/respiratory toxicities				
Hypertension	–	–	–	–
LVEF decrease	–	–	–	–
Dyspnea	24	7	28	9
Pneumonitis	14	4	–	–
Hematologic toxicities				
Anemia	92	12	45	20
Neutropenia	14	0	7	3
Thrombocytopenia	23	1	14	1
Laboratory/metabolic toxicities				
Hyperglycemia	57	15	26	11
Hypercholesterolemia	77	4	24	1
Hypertriglyceridemia	73	<1	27	3
Hypophosphatemia	37	6	–	–
Hyperbilirubinemia	3	<1	–	–
Hypercreatinemia	46	<1	14	3
Increase in AST	21	<1	8	1
Increase in ALT	18	<1	–	–

acute or chronic and is characterized by extreme tiredness and inability to function due to lack of energy. Asthenia includes weakness, lack of energy, and strength. Approximately 50–70 % of mRCC patients complain about fatigue, although only 5–10 % experience severe fatigue interfering substantially with the activities of daily living. Pazopanib appears to have a lower incidence of fatigue as compared to sunitinib and sorafenib although direct prospective comparisons are lacking [2, 30].

It is important to differentiate between drug-related fatigue, cancer-related fatigue, and fatigue related to other conditions (see below). It remains unclear what percentage of fatigue is cancer related and related to other conditions and what is treatment associated, since all types of fatigue are often coexistent in mRCC patients and difficult to differentiate in clinical practice.

To date, the mechanisms for cancer-related and sunitinib-induced fatigue are still poorly understood. A clearer understanding of the molecular mechanisms causing targeted agent-related fatigue would allow more targeted treatment, which might enable better maintenance of drug levels throughout treatment.

In studies, sunitinib-related fatigue was highly variable in both degree and duration. It appeared

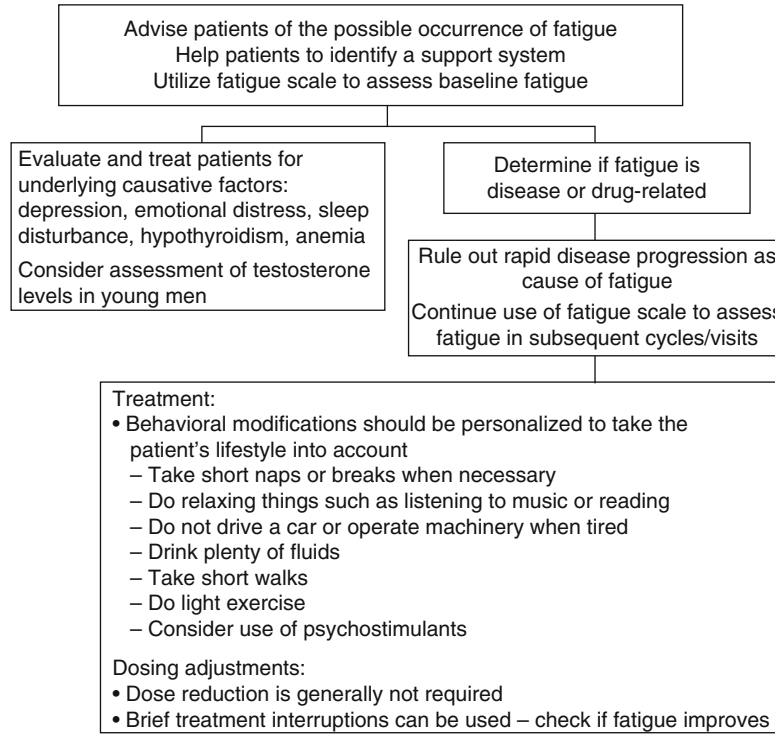

Fig. 22.1 Recommendations for fatigue management

more common in men, particularly in young men, previously treated patients, and patients with repeated treatment interruptions. Typically, it occurred 2–3 weeks after treatment starts, increased in intensity during weeks 3 and 4, and tended to improve during the 2-week-off-treatment period [31]. There did not appear to be an increase in intensity of fatigue/asthenia with increasing number of treatment cycles but rather a decrease. Whether this phenomenon represents an adaptation and learning process by the patient or a true lower incidence remains unclear.

Alternative causes for fatigue should be ruled out before fatigue is attributed to treatment. This includes underlying dehydration, hypothyroidism, hypercalcemia, insomnia, anemia, pain, or depression. Fatigue improves in some patients who have received antidepressants or methylphenidate [32]. Heart failure and decreased left ventricular ejection fraction (LVEF) can also be associated with fatigue.

It is important to educate patients about fatigue, its symptoms, and potential tools to manage fatigue when it presents. Providing patients with written handouts about side effects, their prevention, and side effect management prior to initiating treatment is useful.

Very few evidence-based interventions to treat fatigue exist. Significant fatigue/asthenia interfering substantially with quality of life may be best managed by changes in dose and schedule as discussed above. General principles in the treatment of fatigue are shown in Fig. 22.1. The minimum recommendations for exercise include resistance training or aerobic exercise three times a week for 30 min. Recent randomized trials demonstrate better response in patients using resistance training [33]. The role of psychostimulants and nutritional supplements such as L-carnitine, melatonin, and American ginseng remains controversial with little existing evidence [34–36].

22.4.1 Hypothyroidism

Hypothyroidism has been reported with all VEGFR TKIs. One or more thyroid function test abnormalities developed in up to 85 % of mRCC

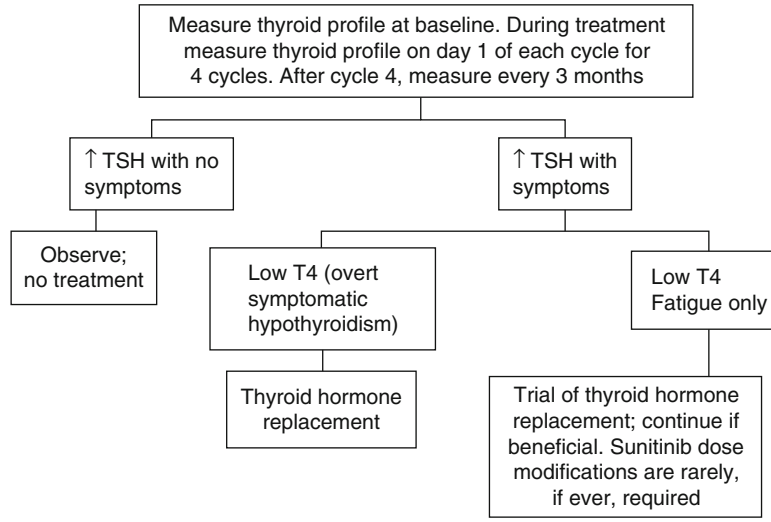

Fig. 22.2 Recommendations for thyroid dysfunction management

patients treated with sunitinib or axitinib [37–41]. There is a substantial discrepancy between incidence rates reported in early prospective trials (lower incidence) and some retrospective series or phase II studies (higher incidence), most likely due to infrequent testing for hypothyroidism in earlier studies, before hypothyroidism was recognized as a common side effect.

The presentation of thyroid dysfunction includes thyroid-stimulating hormone (TSH) elevation only with normal T4 levels (subclinical hypothyroidism), TSH elevation, and low T4 (overt hypothyroidism) that is more likely to be associated with clinical features of hypothyroidism, and even brief episodes of temporary thyrotoxicosis due to thyroiditis, often followed by hypothyroidism, have been described [39, 42, 43].

The exact primary mechanism by which hypothyroidism is caused remains unknown. Several hypotheses have been proposed including direct action of VEGFR TKIs on the VEGFR in the thyroid, induction of a destructive thyroiditis as suggested by the absence of visualized thyroid tissue preceded by TSH suppression, as well as endothelial dysfunction, regression of fenestrated capillaries, inhibition of iodine uptake, and reduced synthesis of thyroid hormone [37, 38, 40, 42, 44].

Hypothyroidism has been reported in patients receiving sunitinib as early as 1–2 weeks after initiation of therapy [42]. TSH tends to improve during the 2-week-off-treatment period. In the sunitinib studies, incidence tended to increase over time, while severity did not seem to increase with cycles. In retrospective series, up to 80 % of sunitinib-treated patients with abnormal thyroid function tests developed symptoms consistent with hypothyroidism such as fatigue, anorexia, edema, fluid retention, or cold intolerance. Thyroid hormone replacement clinically benefited only about 40–50 % of patients treated suggesting additional mechanisms for these side effects [37].

Interestingly, progression-free and overall survivals have been suggested to be improved in patients who experience hypothyroidism compared with euthyroid patients (10.3 months vs. 3.6) indicating that hypothyroidism may be a predictive factor for outcome [42, 45]. A positive correlation between hypothyroidism and improved clinical outcome has also been observed in breast cancer, brain cancer, and head and neck cancers. Importantly, there is no clinical data indicating that treatment of overt hypothyroidism worsens the outcome [45, 46].

Patients undergoing TKI therapy should undergo regular thyroid function monitoring (Fig. 22.2) [38]. All patients showing symptoms of overt hypothyroidism should be treated with thyroid hormone replacement therapy.

Levothyroxine doses should allow normalization of TSH concentrations and resolution of symptoms. Those with asymptomatic subclinical hypothyroidism may be followed without levothyroxine therapy and treated when and if overt hypothyroidism develops. However, subclinical hypothyroidism was recently linked to a significant increase in risk for coronary heart disease events and mortality, indicating that hypothyroidism should be carefully observed and managed [47]. TKI-induced hypothyroidism is generally well manageable, and treatment interruptions or even treatment discontinuation or dose modifications for thyroid dysfunction are usually not necessary. It is important to continue monitoring and thyroxin supplementation after patients come off Rx with TKIs since hypothyroidism does not always resolve off TKI therapy.

22.5 Skin Toxicity

Up to 60 % of patients treated with TKIs and mTOR inhibitors present with some form of skin toxicity including hand-foot syndrome (HFS), hair color changes, skin rash, dry skin, skin discoloration, nail changes, acral erythema, and subungual splinter hemorrhages. Skin toxicity in particular hand-foot syndrome (HFS) and skin rash has been described in up to 60 % of sorafenib-treated patients, approximately 30 % of sunitinib-treated patients, and less than 20 % in pazopanib-treated patients. Skin toxicity typically occurs after 2–4 weeks of treatment [48, 49]. HFS appears to be the most significant of these toxicities, while the other skin toxicities appear well manageable. Preexisting skin conditions should be evaluated and treated prior to TKI or mTOR therapy.

Hand-foot syndrome has been described with all TKIs but with varying frequency. Despite sharing the same spectrum of target receptors with sorafenib, axitinib, and sunitinib, pazopanib appears to be associated with an unexpectedly low risk of HFSR [50, 51].

Symptomatic HFS typically includes painful symmetrical erythematous and edematous areas on the palms and soles, commonly preceded or accompanied by paresthesias, tingling, or numbness. Desquamation can occur in severe cases as well as painful hyperkeratotic areas on pressure points surrounded by rings of erythematous and edematous lesions and painful bullous lesions, blisters, or skin cracking. Areas of pressure are particularly prone to these changes. Preexisting sole hyperkeratosis seems to confer a predisposition for painful sole involvement and functional consequences. TKI-induced HFS can interfere with function in severe cases. TKI-induced HFS is distinct from classic chemotherapy-induced HFS or palmar-plantar erythrodysesthesia.

The exact pathogenesis of this type of HFS is still unknown. Changes can been seen in the epidermal and dermal layers and followed throughout the course of HFS [52, 53]. The most consistent histologic changes are dermal vascular modifications with slight endothelial changes in grade 1–2 HFS and more pronounced vascular alterations with extensive and linear layers of keratinocyte necrosis and intraepidermal cleavage in grade 3 HFS and peribullous lesions [48, 54]. Unproven hypotheses regarding underlying mechanisms include inflammatory infiltration, secretion of the TKI into the eccrine glands resulting in direct toxicity to the skin, as is the case in doxorubicin-associated HFS, and dermal vessel alteration and endothelial cell apoptosis due to direct anti-VEGFR and/or anti-PDGFR [52, 54–57]. Blockade of VEGFR and PDGFR by sunitinib promotes tumor vessel regression by interfering with endothelial cell survival and repair mechanisms [58]. When endothelial survival mechanisms are inhibited in palmoplantar high-pressure areas subjected to repeated trauma through walking, hand washing, and other daily activities, such as the palms and soles, these areas may be unable to repair and thereby acquire the reactive characteristics of HFS [59, 60].

The dose-dependent relationship between TLKIs and HFS also suggests a direct toxic effect of TKIs in HFS pathogenesis [60]. Because an overlap in targets for sorafenib and sunitinib lies in VEGFR and PDGFR inhibition, HFS appears to be an indirect effect of the inhibition of these proangiogenic pathways [53, 60, 61]. The combined inhibition of these receptors appears to be

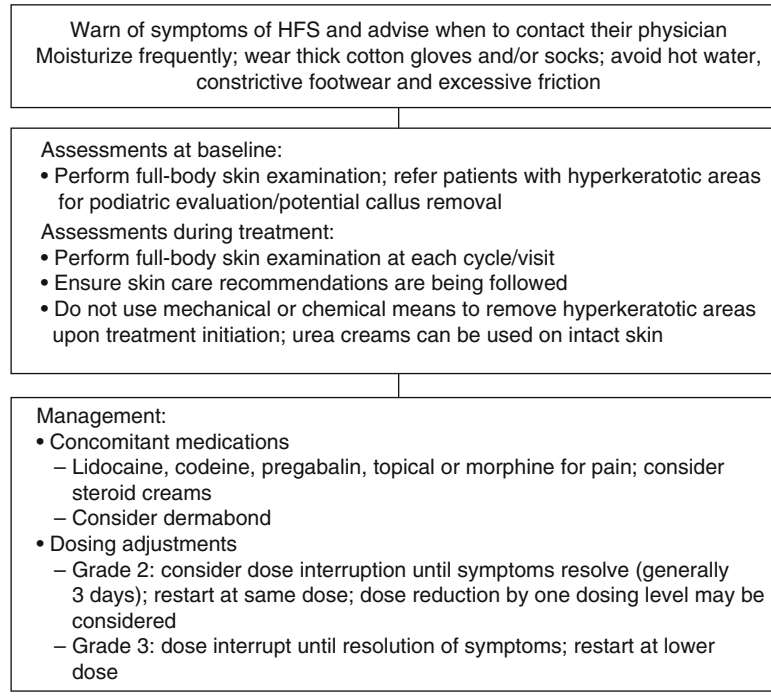

Fig. 22.3 Recommendations for management of hand-foot syndrome

essential because PDGFR (imatinib) or VEGF (bevacizumab) inhibition alone does not result in a similar rate of HFS [62].

Management strategies for HFS are preventative and symptomatic measures (Fig. 22.3). Preexisting calluses and hyperkeratotic areas should be removed prior to treatment. Moisturizers such as simple petroleum jelly-based ointments (e.g., Vaseline®, Aquaphor®) can be applied frequently right from the beginning of therapy. Foot and hand care products (e.g., gel pad inserts, cotton gloves, and clobetasol propionate cream) and medication for pain management can be used for symptomatic patients. Patients should decrease pressure on affected areas, staying off feet when possible and avoiding friction/pressure to hands. Shock-absorbing shoe insoles may be helpful to relieve painful pressure points as well as appropriate footwear and socks to draw moisture from the plantar surface [60]. Topical morphine can be used for patients experiencing severe pain. Steroid creams are also often used, although well-conducted studies are lacking. HFS is not an inflammatory response, but steroid creams may prevent secondary inflammatory processes from taking place. Topical skin adhesives (medical-grade super glue) applied to cracks and painful areas are an option.

Treatment of ≥ grade 2 HFS usually includes the above-discussed measures but often requires dose interruptions, schedule alterations, and if necessary dose reductions as discussed previously. Grade 3 HFS almost always requires dose interruption and frequently subsequent reduction and/or schedule modifications.

For sunitinib therapy, schedule adjustments (e.g., 2 weeks on/1 week off) rather than dose adjustments are often useful as a first step since sunitinib-induced hand-foot syndrome tends to increase over the 4-week period and the pain generally improves quickly (usually within 2–3 days but may take 5 days or longer for higher grades) after removal of the drug. For other TKIs given continuously, brief (2–5 day) dose interruptions may provide substantial benefit while allowing for sustained long-term therapy.

If a patient believed to have HFS does not respond to dose interruption or dose reduction, then other diagnoses must be considered and if necessary treated, including fungal infection or

overgrowth, dyshidrotic eczema, allergic contact dermatitis, and irritant dermatitis.

Generalized erythema and maculopapular or seborrheic dermatitis-like rashes have been reported in approximately 20–60 % of TKI-treated patients with the vast majority being NCI CTCAE grade 1–2 [1, 29, 48, 59, 63]. Skin rashes associated with TKI treatment rarely require dose reduction, and symptoms tend to decrease over time. Dose interruptions may be necessary for higher-grade skin rash (> grade 2), but patients usually can be rechallenged at the same dose level again after recovery to grade ≤1.

Patients should avoid hot showers, use sun protection, and wear loose-fitting cotton clothes. Moisturizing skin creams or lotions, e.g., a colloidal oatmeal lotion, should be frequently applied, in particular after showers and before bedtime [64]. Urea-containing lotions may be helpful, in particular if the skin is very dry. Anti-itch formulas and antidandruff shampoos can be used if itch or scalp discomfort is present [60]. Topical therapies, e.g., steroid creams, may be used for severe cases.

22.6 Oral Toxicity

Oral changes, including sensitivity and taste changes, dry mouth, as well as oral mucosal sensitivity (often referred to as stomatitis/mucositis), occur with varying frequency, in approximately 60 % of patients. Both tyrosine kinase inhibitors and mTOR inhibitors can cause mucositis, but most toxicities are ≤ NCI CTCAE grade 2.

Oral toxicities may occur as early as 7–14 days after the start of therapy. The oral reactions seen during treatment with targeted agents differ from those seen in chemotherapy-induced oral mucositis, which is characterized by local tissue damage and an inflammatory reaction and typically is associated with myelosuppression and mucositis throughout the gastrointestinal tract, causing diarrhea, nausea, and vomiting. TKI-induced oral toxicity in contrast appears to be primarily a "functional" irritation of the mucosa. Patients report a general sensitivity in the mouth which feels sore or they have alterations in taste, but clinical findings are largely normal, and patients do not experience the typical physical signs of a mucositis/stomatitis caused by chemotherapy ("functional stomatitis"). Although mouth ulcerations and aphthous stomatitis may be more frequently seen with mTOR inhibitors, almost all cases are low grade and manageable with supportive measures (grade 3/4 mTOR-associated stomatitis <5 %).

Very few data are available to describe the reactions seen with targeted agents, and the exact mechanism of targeted agent-induced oral toxicities remains unknown. VEGF has been found to be a component of normal human saliva, suggesting that salivary VEGF may play a role in regulating physiologic and pathologic angiogenic and other vascular responses in salivary and mucosal tissues [65].

Treatment for oral side effects is symptomatic only and consists mainly of a modified diet, nutritional consultation, and mouthwashes (Table 22.3). Good oral care should be maintained throughout TKI and mTOR therapy [66]. Oral toxicity can usually be managed symptomatically, and dose adjustments or treatment discontinuation is seldom necessary, while short treatment breaks can be advised for patients with significant discomfort.

Table 22.3 Recommendations for management of oral toxicities

Foods
Avoid hot, spicy, or acidic foods
Eat soft foods that are at room temperature
Cut food into small pieces
Use a straw for drinking liquids
Oral care
Perform routine home oral care
Patients should be instructed to avoid alcohol-containing mouthwashes and consider using a children's toothpaste if toothpaste causes burning
Chlorhexidine and other antimicrobial agents are not warranted as there is no evidence to suggest that oral sensitivity is attributed to gingivitis or periodontal disease
Symptomatic relief:
Magic mouthwash containing equal parts of 2 % viscous lidocaine, diphenhydramine, and bismuth subsalicylate or aluminum/magnesium hydroxide

22.7 Diarrhea

Diarrhea occurs in approximately 30–50 % of patients treated with TKIs, but grade 3/4 toxicity is rare and observed in only 3–8 % of cases. Some degree of diarrhea is often the main toxicity remaining when other common toxicities have been controlled with dose/schedule changes. In contrast to chemotherapy-induced diarrhea, which is usually continuous, TKI-induced diarrhea can occur irregularly with days of diarrhea mixed in with days of normal bowel movements. The incidence of diarrhea associated with mTOR inhibitors is lower (<20 %) with severe grade 3/4 diarrhea being very rare (1 %).

The underlying pathogenesis for TKI-induced diarrhea is not known. Bowel mucosa changes consistent with ischemic colitis have been reported after treatment with other VEGF-interacting agents, in particular bevacizumab [67].

Grade 1/2 diarrhea can usually be well managed by symptomatic measures including oral hydration, oral antidiarrheal agents as needed such as loperamide, and dietary changes. Patients can be advised to drink plenty of liquids (but in small amounts at a time, avoiding drinking fluids with meals and for 1 h after), eat and drink often in small amounts, and avoid spicy foods, fatty foods, caffeine, and high-fiber foods. Stool softeners and fiber supplements as well as magnesium-containing antacids should be discontinued.

Dose reductions are rarely necessary for grade 1 and 2 toxicity, while treatment should be interrupted for grade 3 or 4 diarrhea until diarrhea is grade ≤1 or has returned to baseline. Upon rechallenge, dose or schedule changes are frequently required to control diarrhea in subsequent cycles. Diarrhea usually resolves quickly during treatment breaks.

A number of other gastrointestinal side effects including taste changes, dry mouth, nausea, vomiting, and indigestion occur with varying frequency (10–30 %). Dose adjustments or interruptions are seldom necessary. Anorexia is found in about 10–20 % of patients but rarely exceeds grade 2. Although anorexia rarely requires dose modifications, underlying causes should always be investigated, in particular a potential relationship to coexisting hypothyroidism and other gastrointestinal toxicities. Patient education regarding nutrition and consultation with a dietician is recommended.

The emetogenic potential of TKIs and mTOR inhibitors is low. Less than 5 % of patients experience grade 3 or 4 vomiting/nausea and only 10–30 % grade 1–2 [1, 7, 68, 69]. Common antiemetics can be used to relieve or prevent nausea and vomiting. However, particular care should be used when combining targeted agents with antidopaminergic agents such as domperidone or 5HT3 antagonists, such as granisetron, ondansetron, and dolasetron, since they have been associated with QT/QTc interval prolongation and/or torsade de pointes, a potential side effect also associated with TKI therapy.

H2-blockers are recommended for the treatment of heartburn and indigestion.

22.8 Hematotoxicity

Myelosuppression has been observed with both TKIs and mTOR inhibitors. Sunitinib induces neutropenia and thrombocytopenia in about 20 % of non-Asian patients. Only 5–8 % of patients develop grade 3/4 neutropenia or thrombocytopenia, and very few cases of neutropenic fever have yet been reported. Treatment modifications should only be considered for grade 3 or 4 toxicity and/or clinical symptoms such as neutropenic fever or bleeding signs or for severe anemia. Blood counts usually recover quickly during treatment breaks. Hematopoietic growth factors are rarely required. Ethnic background appears to impact on the incidence of hematotoxicity. Recent data suggest a significantly higher incidence of myelotoxicity, in particular neutropenia and thrombocytopenia, in Asian patient populations [70].

While the exact mechanism of hematotoxicity associated with targeted agents remains to be elucidated, inhibition of c-kit by various TKIs, e.g., sunitinib, may play a role. C-kit has a well-established role in hematopoiesis and melanocyte differentiation [71].

22.9 Hypertension

Hypertension is a class effect of angiogenesis inhibitors interfering with the VEGF receptor [11, 72–75] but has only been very rarely described with mTOR inhibitors. Hypertension develops in up to 60 % of patients although severe grade 3 or 4 hypertension is rare (<10 %). The exact pathogenesis by which angiogenesis inhibitors induce hypertension is not yet known. It has been speculated whether TKIs may exert hypertensive effects directly at the level of the vasculature through processes such as vascular rarefaction, endothelial and microvascular dysfunction, and/or altered nitrous oxide metabolism [72, 76–78].

The development of hypertension has also been shown, similar to hypothyroidism, to be associated with an improved outcome and may therefore serve as a predictive marker for response [79]. Patients should undergo a formal risk assessment for potential cardiovascular complications including standardized blood pressure measurements on at least two separate occasions, thorough history and examination to assess specific cardiovascular risk factors, and laboratory studies examining conditions predisposing patients to cardiovascular morbidity such as fasting glucose and lipid levels and serum creatinine level. Preexisting hypertension must be controlled before initiation of antiangiogenic therapy. Patients with preexisting hypertension are generally more likely to develop further elevation in blood pressure when receiving anti-VEGF therapy. All patients should be monitored for hypertension throughout treatment but in particular frequently during the first cycles. Daily blood pressure (BP) monitoring in the home setting and BP data kept in a patient diary is suggested in this patient population during the first two to three cycles since hypertension can develop within days after initiation of antiangiogenic therapy.

Hypertension should be graded either according to the National High Blood Pressure Education Program categories or the new CTCAE version 4 Hypertension scale which has now been aligned with US National High Blood Pressure Education Program categories to improve communication among oncologists, cardiovascular medicine specialists, and primary care physicians [75].

Since larger prospective studies in patients with anti-VEGF therapy-induced hypertension are lacking, treatment should be initiated based on current hypertension guidelines for the general patient population which are available from different hypertension societies such as the Canadian Hypertension guidelines or the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines [80, 81]. Most commonly used antihypertensive agents in previously normotensive patients include angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), dihydropyridine calcium channel blockers (CCBs) such as amlodipine, and beta-blockers. The treatment objective is blood pressure normalization with resting rate <140/90 mmHg.

Until more clinical data become available, non-dihydropyridine calcium channel blockers such as diltiazem and verapamil should be avoided, as they are known CYP3A4 inhibitors. Other antihypertensive drugs may also interact with cytochrome P450, and potential drug interactions have to be considered. Consultation with a hypertension specialist should be obtained promptly if blood pressure control cannot be reached. Active control of hypertension should allow patients to tolerate the highest effective dose of VEGF pathway inhibitor therapy and benefit from the tumor growth control for the longest period, improving quality and length of life.

Temporary suspension of treatment is recommended for patients with severe hypertension (>200 mmHg systolic or >110 mmHg diastolic). Treatment may be resumed once hypertension is controlled.

Therapy for hypertension is often only required during the therapy phase and may be discontinued when patients are off drug. The effect of anti-VEGF agents on blood pressure is dose dependent, but generally, hypertension can be well controlled with proper antihypertensive medication and dose reductions, or even treatment discontinuations are very rarely necessary in particular in previously normotensive patients.

22.10 Cardiac Toxicity

Left ventricular dysfunction, which manifests as a decrease in left ventricular ejection fraction (LVEF), is the main cardiac side effect of TKIs, whereas arrhythmias including bradycardia and PR and QT prolongation have been rarely observed (<1 %). Cardiac toxicity with mTOR inhibitors is rare (<1 %). TKI-induced cardiac failure and left ventricular dysfunction rates vary greatly in the literature ranging from as low as 2–3 % up to 33 % in some smaller studies, but symptomatic ventricular dysfunction (CTC grade 3/4) occurs rarely (<3 %). A recently published meta-analysis examining the incidence and risk for congestive heart failure in patients treated with sunitinib suggested an overall incidence for all-grade and high-grade CHF in sunitinib-treated patients of 4.9 % and 1.8 %, respectively [82]. The differences in observed incidence among the studies may stem from differing methodologies for study design, patient selection, and ascertainment of cardiotoxicity with varying frequency of cardiac monitoring or from different biologic effects of different TKIs on the heart [83]. The true risk of cardiotoxicity of TKIs and mTOR inhibitors is not known because prospective thorough clinical assessments of left ventricular function have not been done in any of the large trials although smaller institutional series indicate cardiac abnormalities in up to 33 % of patients [83].

Cardiotoxicity is thought to develop due to on- and off-target effects and inhibition of multiple kinase, some of which may also be essential for cardiomyocyte homeostasis and the function of the heart. Additional stress through other effects such as hypertension can be particularly problematic in patients with an already compromised cardiac reserve or advanced coronary artery disease [84, 85]. However, cardiotoxicity has been observed in patients with and without TKI-induced hypertension suggesting that additional mechanisms may be responsible [86].

A number of studies in various mouse models have shown that angiogenesis (which is mediated in the heart by both veGFR2 and PDGFRβ, targets of sunitinib) is key to maintaining cardiac homeostasis in the setting of a pressure load or ischemia [87, 88].

No head-to-head cardiotoxicity studies have been conducted with anti-VEGFR TKIs, but the frequency of cardiotoxicity appears to vary between different TKIs despite a similar inhibition profile [89]. Although this may be due to the potency of inhibiting VEGFR, the difference suggests the possibility of additional off-target effects such as sunitinib-induced inhibition of AMPK, a kinase essential for increased energy generation and decreased energy utilization in cardiomyocytes [90, 91].

Recent preclinical studies have demonstrated that, although pazopanib, sunitinib, and sorafenib have a similar tyrosine kinase inhibition profile, they differ in their effects on functional and structural parameters of myocardial toxicity with pazopanib showing the least toxicity [92]. This appears consistent with clinical data to date suggesting a very low incidence of cardiotoxicity with pazopanib. However, clinical data on the frequency of pazopanib and axitinib toxicity are limited thus far, and experiences in broader, unselected populations are lacking.

Patients who present with cardiac risk factors or a history of cardiac events (e.g., acute coronary syndrome, arterial bypass graft, symptomatic congestive heart failure (CHF), stroke, or pulmonary embolism) should be monitored for clinical signs and symptoms of CHF and evaluated for decreased LVEF while receiving TKIs, with echocardiography or MUGA done at baseline and at intervals during therapy. Blood pressure should be monitored more frequently in patients with a history of CHF since hypertension can accentuate the clinical symptoms of CHF. In patients without cardiac risk factors, a baseline evaluation of ejection fraction may be considered with subsequent screening every 3–6 months as clinically indicated.

In contrast to anthracycline-induced cardiomyopathy, patients with TKI-induced cardiac dysfunction generally respond well to standard heart failure management for nonischemic cardiomyopathy. Treatment of TKI-induced heart failure includes withholding the agent while heart failure management is instituted, aggressive treatment of TKI-induced hypertension, medical therapy including angiotensin-converting enzyme

inhibitors, and diuretics. Beta-blockers may be initiated as well but may contribute to fatigue and thus not be well tolerated. Clinically symptomatic CHF requires treatment interruption and initiation of cardiac therapy. Refractory CHF with fatal outcomes has rarely been reported in trials of antiangiogenic agents. Recent clinical studies suggest reversibility of TKI-induced cardiotoxicity and ventricular dysfunction improved after cessation of the anti-VEGF agent and with proper cardiac therapy in most patients (type II cardiotoxicity) [93, 94]. The recovery of function and the absence of irreversible changes seen on the endomyocardial biopsy of patients treated with targeted therapy suggest that cardiac dysfunction may at least be partially reversible [86, 95]. Patient with asymptomatic or even symptomatic heart failure may therefore be rechallenged after recovery of heart function, in particular if alternative treatment options are limited and patients derived a good benefit from treatment [93].

In patients with LVEF <50 % and >20 % below baseline, temporary interruption and/or dose reduction of TKI treatment can be considered and heart failure therapy initiated regardless of clinical evidence of CHF. Very little is currently known about the long-term sequelae of TKI-induced cardiovascular dysfunction.

Caution is advised if QT/QTc or PR prolonging agents are combined with sunitinib due to potential drug interactions.

22.11 Pneumonitis

Drug-related noninfectious pneumonitis is a class-effect toxicity of mTOR inhibitors and has been reported with both everolimus and temsirolimus. Radiographic changes consistent with pneumonitis with or without symptoms have been reported in 25–40 % of kidney cancer patients treated with temsirolimus and everolimus [6, 7, 96, 97]. Initial studies including the pivotal phase III studies for temsirolimus and everolimus underestimated the incidence of pneumonitis. Recently published blinded, independent, retrospective radiological reviews of the pivotal randomized phase III mTOR inhibitor trials demonstrated an up to 29 % incidence of temsirolimus and a 39 % incidence of everolimus-associated pneumonitis [96–98]. Radiographic changes consistent with mTOR-related pneumonitis are not always associated with clinical symptoms. Only approximately 30–40 % of these patients are symptomatic, mostly with dry cough and dyspnea. Systemic symptoms of fever and fatigue have been reported in some cases as well. Onset of pneumonitis usually occurs within the first 2–4 months in the majority of the patients (60 %) with ground-glass opacities (71 %) and patchy air space consolidation (62 %) being the most common radiological findings at presentation [96, 97]. Chest CT scans are the recommended method to detect mTOR inhibitor-induced pneumonitis, since chest x-rays are less sensitive than CT scans in detecting asymptomatic radiographic findings or clinical pneumonitis. Pulmonary function tests usually show a restrictive pattern or an isolated reduction in diffusing capacity.

The pathophysiology of mTOR inhibitor-induced pneumonitis remains unclear. Radiographic diagnosis and evaluation of noninfectious pneumonitis can be challenging and should not be confused with progressive pulmonary disease or infection. New lung lesions and ground-glass pattern with or without consolidation should be carefully examined for the presence of pneumonitis versus progressing disease or infection.

All patients treated with mTOR inhibitors should be warned to promptly report symptoms such as dyspnea or dry cough. Suggested management recommendations of noninfectious mTOR-induced pneumonitis are empiric and should rely on combined radiographic and clinical assessments. Treatment is dependent on the severity of the associated symptoms, with limited symptoms allowing for continuation of therapy, patients with moderate symptoms potentially benefiting from interruption, and severe symptoms warranting a combination of mTOR discontinuation and corticosteroid therapy (Table 22.4). Even in cases of severe noninfectious pneumonitis, it may be feasible to restart therapy at a reduced dose depending on patient-specific considerations, in particular in patients without

Table 22.4 Management recommendations for mTOR inhibitor-induced pneumonitis (mod. Acc White et al. [97])

Grade	Symptoms/radiographic changes	Treatment
1	Asymptomatic, radiographic changes only	Establish absence of symptoms
		Repeat chest CT q 2–3 cycles
		Caution patient to immediately report respiratory symptoms
		No specific therapy
		Continue treatment without change but with close observation for development of symptoms
		No dose adjustment/treatment interruption required
		Exceptions could be considered, e.g., underlying ILD or if the infiltrates are extensive
2	Symptomatic, medical intervention indicated, limiting instrumental activities of daily living	If clinically indicated, tumor progression, infection, or other causes of radiographic infiltrates/respiratory symptoms, such as fluid overload or pulmonary embolus, should be excluded
		Consider pulmonary function tests
		Temporary treatment interruption or dose reduction until grade ≤1 (usually for 7–10 days)
		Short course (8–10 days) of prednisone 20 mg/day if symptoms are troublesome or if they persist despite treatment interruption/dose reduction
		Restart treatment at the same dose (preferred) or one dose level below at the physician's discretion
3	Severe symptoms and limiting self-care activities of daily living, oxygen indicated	If clinically indicated, tumor progression, infection, or other causes of radiographic infiltrates/respiratory symptoms, such as fluid overload or pulmonary embolus, should be excluded
		Pulmonary function tests ± bronchoscopy with bronchioalveolar lavage and biopsy
		Hold mTOR inhibitor until grade ≤1
		Short course (8–14 days) of prednisone 20–30 mg/day if respiratory symptoms are mild to moderate
		Short course (8–14 days) of high-dose prednisone (e.g., 1 mg/kg) for patients with severe respiratory distress – taper as medically indicated
		If symptoms resolve promptly, restart treatment one dose level below the previous dose level in selected cases
4	Life-threatening respiratory compromise and urgent intervention indicated (e.g., tracheotomy or intubation)	Rule out tumor progression, infection, or other causes of radiographic infiltrates/respiratory symptoms, such as fluid overload or pulmonary embolus
		Pulmonary function tests ± bronchoscopy with bronchioalveolar lavage and biopsy
		Discontinue mTOR inhibitor permanently
		Course (8–14 days) of high-dose prednisone (e.g., 1 mg/kg) – taper as medically indicated

alternative treatment options. Symptoms usually improve and disappear quickly during treatment breaks. Pneumonitis appears to be dose dependent in some individuals who tolerate lower doses, and treatment with corticosteroids usually leads to rapid improvement of symptoms. Although there are clinical and pathological similarities of pneumonitis with all mTOR inhibitors, relapse does not always occur after switching to another agent [99].

22.12 Bleeding

Bleeding events and tumor hemorrhage have been reported in approximately 20–25 % of patients receiving TKIs for mRCC [100]. Epistaxis was the most common hemorrhagic side effect reported; less common bleeding events included rectal, gingival, upper GI, genital, and wound bleeding. Treatment-related tumor hemorrhage has been rarely observed (<2 %). Severe grade 3 and 4 bleeding incidents are very rare (<2 %) [1–3, 100]. Assessment of hemoptysis should include serial complete blood counts and physical examination. Temporary discontinuation of therapy may be considered until the cause of hemorrhage is determined. A dose discontinuation is usually not indicated for mild to moderate bleeding episodes and may only be considered in cases of severe or uncontrollable bleeding.

22.13 Laboratory Abnormalities/ Metabolic Changes/Liver and Renal Toxicity

A number of laboratory abnormalities have been described associated with TKIs and mTOR inhibitors. Laboratory abnormalities rarely require intervention. It may be difficult to differentiate between treatment-induced and disease-induced changes. Tyrosine kinase inhibitors can induce elevations of amylase and lipase in 30–50 % of cases (all CTC grades), but no case of TKI-induced pancreatitis has yet been reported. Electrolyte disturbances can usually be managed with oral supplementation. Another frequently observed side effect of angiogenesis inhibition is renal toxicity. Bevacizumab frequently induces proteinuria, while a grade 1–2 rise in creatinine levels was rather common in the phase 3 trials with TKIs. Increases in creatinine levels, even severe renal failure, only occasionally warrant treatment interruption or dose reduction, as the pharmacokinetics of biologic agents are rarely affected by kidney failure [101, 102]. Serum creatinine should be carefully monitored during therapy with targeted agents in particular in patients with preexisting renal impairment.

Hyperglycemia and hyperlipidemia are class effects of mTOR inhibitors resulting from mTOR's involvement in intracellular glucose and lipid regulation but have also infrequently been reported during TKI therapy. Increases in blood glucose levels can be observed in both diabetic and nondiabetic patients. Approximately 25–50 % of patients develop abnormal glucose levels with 10–15 % being grade 3/4 [6, 7]. Preexisting diabetes confers a higher risk to develop hyperglycemia, and preexisting hyperglycemia should be controlled prior to initiation of mTOR or TKI therapy.

The management of TKI/mTOR inhibitor-induced hyperglycemia should be based on existing standard diabetes management guidelines such as the "the International Diabetes Federation and the American Diabetes Association" guidelines and includes oral hypoglycemic agents, e.g., metformin or rosiglitazone and/or insulin therapy. Educating patients about the signs and symptoms of hyperglycemia is important [103].

Abnormalities in lipid metabolism including both hypertriglyceridemia and hypercholesterolemia have been observed in 27 % and 71 % of patients treated with temsirolimus and everolimus, respectively, in the pivotal trials [6, 7]. However, less than 5 % were grade 3/4. Lipid levels should be assessed prior to treatment and therapy initiated if necessary. Monitoring of lipid levels during therapy with mTOR inhibitors is recommended. No definitive therapeutic recommendations have been developed, and treatment of hyperlipidemia should follow existing guidelines, e.g., American College of Physicians and the National Cholesterol Education Program [104]. HMGCoA inhibitors (i.e., statins) are the preferred option if active treatment is indicated. However, it is important to note that the clinical management of hypercholesterolemia and hypertriglyceridemia in patients with advanced RCC represents a different challenge due to their limited life expectancy. Existing treatment guidelines estimate the morbidity from hyperlipidemia, e.g., probability of a CV event over a period of many years and in relationship to many other risk factors and the morbidity from short-term hyperlipidemia as in mRCC patients is thought to be very small.

Hepatotoxicity manifested as increases in serum transaminases (ALT, AST) and bilirubin has been observed with all TKIs and in particular with pazopanib [105, 106]. ALT elevations greater than three and eight times the upper limits of normal (ULNs) have been observed in 14 % and 4 % of all patients treated with pazopanib, respectively. Concurrent elevations of ALT greater than three times ULN and bilirubin greater than two times ULN occur in 1 % of pazopanib patients. Fatal hepatic failure has been reported in 2 of 977 (0.2 %) pazopanib patients evaluated [107]. Severe hepatic dysfunction has been rarely reported after treatment with other TKIs [108].

Hepatic function should therefore be determined prior to initiation of therapy and monitored throughout the duration of pazopanib therapy. Typically, most transaminase elevations occur within the first 18 weeks of treatment making frequent testing of hepatic function within the first 4 months of therapy mandatory, e.g., at baseline and every 3–4 weeks.

Pazopanib may be continued in cases of isolated transaminase elevations of three to eight times ULN, but hepatic function should be monitored more frequently [107]. Patients with transaminase elevations greater than eight times ULN should have their treatment interrupted until ALT returns to grade 1 or baseline. Pazopanib may be reinitiated at a reduced dose of 400 mg daily with close monitoring of hepatic function, e.g., weekly if the patient derives benefit from pazopanib therapy. Pazopanib should be permanently discontinued if, after reinitiation of pazopanib, transaminase increases again to greater than three times ULN [107]. Pazopanib must be permanently discontinued in patients experiencing transaminase elevations greater than three times ULN concurrently with an increase in total bilirubin greater than two times ULN.

Pazopanib inhibits UGT1A1, an enzyme involved in the metabolism of bilirubin, and pazopanib-induced hyperbilirubinemia has been associated with a polymorphism of the gene for UGT1A1 found in patients with Gilbert's syndrome. Mild elevation of indirect bilirubin without other potential causes may be a benign manifestation of Gilbert's syndrome, and a treatment interruption may not be required [109].

Conclusions

TKIs and mTOR inhibitors have demonstrated significant efficacy in the treatment of mRCC. The unique toxicities associated with targeted therapies pose a new challenge for the healthcare team. It has become clear that effective toxicity management is a key requirement for achieving the maximum benefit for the patient, since continuous therapy and dose intensity are important and dose reductions should be avoided whenever possible.

Most toxicities are typically mild to moderate in intensity and are generally manageable with standard medical interventions, without treatment discontinuation or permanent dose reduction. However, the accumulation of several lower-grade side effects can represent a substantial challenge and often requires dose/schedule changes and in some cases treatment termination. Elderly patients appear to derive a similar benefit as younger patients and without substantially increased toxicity [68, 110, 111].

Patient education about potentially bothersome side effects is an important part of toxicity prevention and treatment. Effective communication within the healthcare team and with the patients is key to successful toxicity management in patients with mRCC.

Little is known about the mechanisms leading to these side effects, which makes causal treatment of side effects impossible. Their exploration remains a priority in order to improve management.

The impact of pharmacogenomics on the incidence and severity of side effects is poorly understood. Recent evidence has suggested that heterogeneity in toxicity and efficacy among patients receiving anti-VEGF therapy could be at least partially explained by genomic variability, including single-nucleotide polymorphisms, providing a possible explanation for the differences in toxicity frequencies between Asians and non-Asians in this analysis. Female gender, age, and low body surface area have

also been reported to predict for severe side effects. A better understanding of genetic and nongenetic determinants of targeted-therapy-associated toxicity should help to optimize drug treatment in individual patients.

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Emerging Agents in Renal Cell Carcinoma

23

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Support: Dr. Pal's efforts are supported by CBCRP 15IB-0140 (California Breast Cancer Research Program Junior IDEA Award) and NIH K12 2K12CA001727-16A1.

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Key Points

- Though seven agents for mRCC have been approved over the past 5 years, the disease remains largely incurable.
- The recently approved agents fall within one of two generalized categories (VEGF-directed therapies or mTOR inhibitors); moving forward, the research community will need to examine novel therapeutic targets and approaches.
- AGS-003 is a dendritic cell vaccine that has showed encouraging activity in combination with sunitinib in a phase II

P.N. Lara Jr., E. Jonasch (eds.), *Kidney Cancer: Principles and Practice*,
DOI 10.1007/978-3-319-17903-2_23

study largely including patients with intermediate and poor risk.
- IMA901 is a vaccine comprised of tumor-associated peptides that has shown encouraging activity in a phase II study; clinical activity appears to correlate with immune response.
- Several agents are in development that inhibit angiogenesis without direct abrogation of VEGFR signaling; for instance, CVX-060 and AMG-386 disrupt the Ang-1/2/Tie-2 signaling axis.
- Several novel therapies expand beyond the current paradigm of antiangiogenesis or immunotherapy for mRCC – these include dovitinib (a dual VEGFR/FGFR1 inhibitor), XL184 and GSK089 (dual c-MET/VEGFR2 inhibitors), and AMG-102 (a monoclonal antibody directed at HGF).
- Preclinical studies have outlined a putative role for numerous moieties (i.e., JAK2, ALK, Stat3, etc.) in RCC pathogenesis; many of these represent potential therapeutic targets.

23.1 Introduction

Within the past decade, a marked shift has occurred in the treatment paradigm for metastatic renal cell carcinoma (mRCC). Previously, immunotherapy (i.e., interleukin-2, IL-2, and interferon-alpha, IFN-alpha) represented the principal treatment modality for metastatic disease [1–3]. Today, the therapeutic algorithm is populated with six additional targeted therapies, each approved on the basis of randomized, phase III trials [4–10]. While the availability of a wide array of treatment options is no doubt reassuring to the patient, the oncologist may recognize multiple areas of mechanistic overlap. Four of the approved agents (sunitinib, sorafenib, pazopanib, and bevacizumab) antagonize signaling via the vascular endothelial growth factor receptor (VEGF) pathway, while the two remaining agents (temsirolimus and everolimus) both inhibit the mammalian target of rapamycin (mTOR) [11]. Although the cumulative effect of these therapies has been to improve historical benchmarks for clinical outcome, the fact remains that these treatments are rarely curative [12]. Moving ahead, the research community will have to look toward novel therapeutic strategies that go beyond targeting the VEGF and mTOR signaling axes. The current chapter will outline such approaches that are currently under clinical investigation.

23.2 Novel Immune Strategies

23.2.1 Vaccine Therapy

Several vaccine-based approaches have been devised for the use in mRCC. Akin to sipuleucel-T (recently approved for treatment of metastatic castration-resistant prostate cancer), AGS-003 represents an autologous dendritic cell (DC) vaccine [13]. The methodology for generating this vaccine differs greatly, however. Candidates for AGS-003 therapy must have had fresh viable tumor tissue from either a primary or metastatic site to facilitate vaccine production [14]. RNA from tumor issue is isolated, and this RNA is then electroporated into autologous DCs derived from leukapheresis. Presumably, RNA that is translated by the DC will yield peptide sequences that will stimulate cytotoxic T-cells.

A phase II study utilizing the combination of sunitinib with AGS-003 in newly diagnosed mRCC was recently reported [15]. Patients were required to have either a primary tumor amenable to nephrectomy or a metastatic site amenable to metastasectomy as a source of fresh tissue. Sunitinib was administered at standard doses (50 mg daily; 4 weeks on, 2 weeks off), and AGS-003 was injected at regular intervals in two phases. In an induction phase, AGS-003 was injected every 3 weeks for a total of five doses (concurrent with sunitinib). In a maintenance phase, the vaccine was administered every 3 months until the time of disease progression. The primary endpoint of this study was objective response rate (RR).

Ultimately, a total of 22 patients were treated [15]. No grade ≥3 adverse events (AEs) were attributed to AGS-003; instead, the side effect profile of the combination regimen appeared to be similar to that of sunitinib alone. Of 16 evaluable patients, 4 patients (25 %) had a partial response (PR), while 8 patients (50 %) exhibited stable disease (SD). The progression-free survival (PFS) associated with the regimen was 11.2 months. Notably, no patients were categorized as having good-risk disease by MSKCC criteria; instead, 16 patients were noted to have intermediate-risk disease, while the remaining 6 patients had poor-risk disease. In the intermediate-risk population, a PFS of 15.1 months was observed, as compared to 6.0 months in the poor-risk population. These results compare favorably to the PFS observed among subgroups stratified by MSKCC risk group in the pivotal phase III trial of sunitinib therapy [8]. Given the limited toxicity and encouraging efficacy of the sunitinib/vaccine combination, a phase III study (the ADAPT trial) was launched, comparing sunitinib monotherapy to sunitinib with AGS-003 in patients with de novo metastatic disease [16]. Recent estimates suggest that the study is roughly midway in accrual of roughly 450 patients.

Other vaccine-based approaches have been devised for the use in mRCC. As one prominent example, IMA901 represents a composite of tumor-associated peptides (TUMAPs) [17]. These peptides represent HLA class II ligands that are preferentially expressed in tumor tissue as compared to normal parenchyma. Recently, data from a randomized, phase II effort examining IMA901 was reported [18, 19]. The protocol accrued human leukocyte antigen (HLA) A02-positive patients with metastatic RCC after failure of cytokines or VEGF-TKIs. Sixty-eight patients were randomized to receive IMA901 with GM-CSF with or without a single infusion of cyclophosphamide therapy (300 mg/m^2) preceding vaccine administration. Seventeen vaccinations with IMA901 were rendered for each patient over a 9-month period. The primary endpoint in this study was 6-month disease control rate (DCR).

In 40 patients previously treated with cytokines, a DCR of 31 % at 6 months was achieved [18]. In contrast, in 28 patients who had previously received TKI therapy, DCR was 12 %. Notably, at the time of most recent report, a median overall survival (OS) had not been reached in patients with cytokine pretreatment. The immune response to IMA901 was documented; those patients with a superior immune response were noted to have improved OS ($P=0.019$). Akin to the clinical development of AGS-003, a phase III trial has commenced that will compare sunitinib monotherapy to the combination of sunitinib with IMA901. The study has completed accrual, and results are highly anticipated [20].

Oudard et al. have recently reported initial data for a MUC1-based vaccine for RCC [21]. MUC1 represents a cell surface glycoprotein that may inhibit cellular interactions, thereby limiting contact inhibition and promoting tumor growth [22]. In clear cell mRCC, increased MUC1 expression is associated with poorer survival [23]. TG4010 represents a construct comprised of modified vaccinia virus of the Ankara strain (MVA) expressing both IL-2 and MUC1 antigen [24]. A phase II study was conducted in patients with mRCC with documented MUC1 expression (positive staining in >50 % of cells) [21]. Patients may not have had any prior therapy for metastatic disease and were required to have clear cell histology. TG4010 was administered as a subcutaneous (SQ) injection weekly for 6 weeks and then every 3 weeks until disease progression. At that point, cytokine therapy (low-dose IL-2 and IFN-α) was concomitantly administered with TG4010. Of 37 patients enrolled, only 27 patients (73 %) were evaluable. Of the 27 patients who received TG4010 alone, 5 patients (18 %) had SD lasting >6 months. Of 20 patients who proceeded to receive immunotherapy, 6 patients (30 %) had SD for >6 months. Although toxicities associated with TG4010 were limited, it remains to be seen how this modest efficacy data will translate into further clinical development of the agent.

Several other vaccine-based strategies are currently in development. For instance, MGN1601 is a cell-based tumor vaccine that contains two components: (1) a DNA-based molecule that activates TLR-9 and (2) modified allogeneic cells infected with vectors encoding

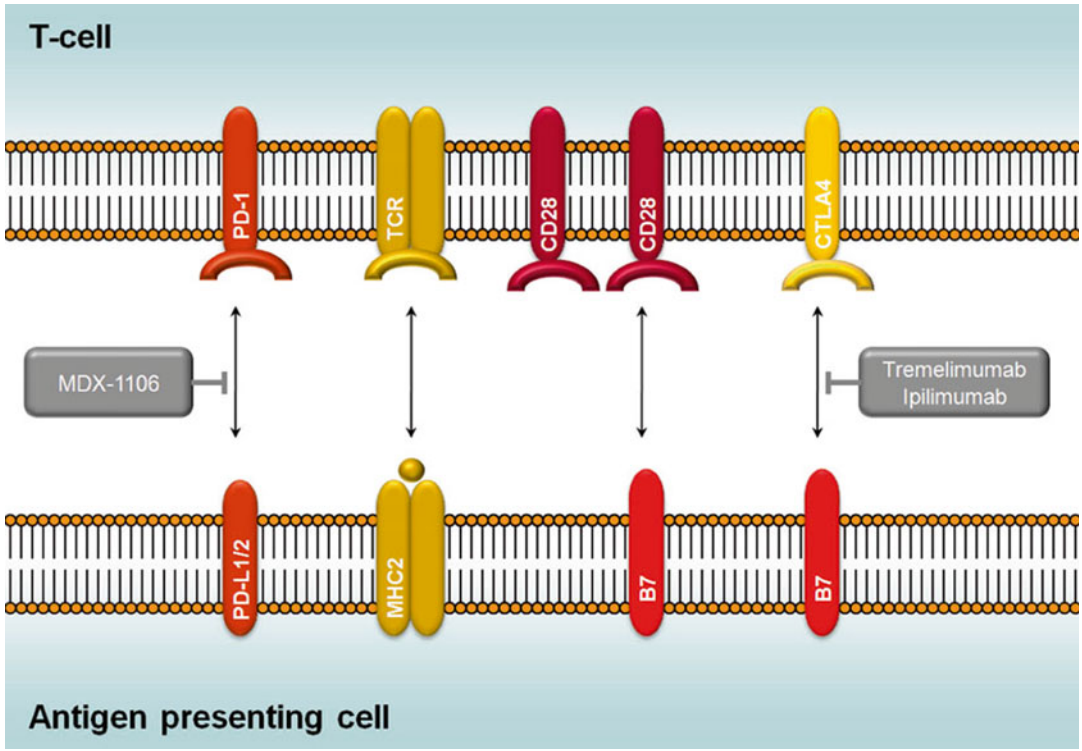

Fig. 23.1 Novel immune agents targeting immune signaling. MDX-1106 is a monoclonal antibody with affinity for PD-1. Binding of PD-1 on the T-cell surface to PD-L1/2 on the antigen-presenting cell (APC) leads to induction of T-cell anergy. In contrast to MDX-1106, tremelimumab and ipilimumab bind to CTLA4, preventing its interaction with B7 and promoting T-cell proliferation

IL-7, CD80, GM-CSF, and CD154 [25]. Murine analogues of the vaccine have demonstrated efficacy, and a phase I/II study including patients with mRCC is underway [26]. Also under development are techniques that utilize ex vivo expansion of immunoreactive cells. Bennouna et al. reported a phase I effort examining an ex vivo expansion of γ9δ2 T-cells with IPH1101-Phosphostim 200 and IL-2 [27]. γδ T-cells demonstrate potent antitumor effects in preclinical models of RCC but typically represent a small proportion (<10 %) of the T-cell population. The expansion technique generates a stimulated product in which >95 % of the cells are of the γδ subtype [28]. In a series of ten patients, expanded T-cells were infused alone and then combined with low-dose subcutaneous IL-2. The agent demonstrated limited toxicity, and six patients (60 %) had SD as a best response. Further data regarding this approach is eagerly anticipated.

23.2.2 Programmed Death-1 (PD-1) Inhibition

PD-1 inhibition enhances the antitumor activity of T-cells [29]. The activation of a T-cell is dependent upon two specific interactions (Fig. 23.1). First, the T-cell receptor (TCR) must interact with the peptide antigen-major histocompatibility complex (MHC) on the antigen-presenting cell (APC). Second, there is a required interaction between co-stimulatory molecules – specifically, CD28 expressed on the T-cell surface interacts with B7 on the APC. Concomitant with T-cell activation is expression of PD-1, which interacts with ligands PD-L1 and PD-L2 on the surface of APCs. Ligand association with PD-1 leads to downregulation of T-cell function. From a clinical standpoint, expression of PD-L1 occurs in a constitutive fashion in patients with RCC and is associated with a more aggressive disease course [30].

Notably, PD-1 and PD-L1 inhibition is the subject of another chapter in the current manuscript, and will therefore not be explored in detail here. These agents have strong potential to impact the therapeutic landscape of mRCC. As one example, the PD-1 inhibitor nivolumab has shown impressive clinical outcome in several recently reported monotherapy trials [31–33]. An ongoing phase III study will compare nivolumab to everolimus in patients with prior exposure to VEGF-directed therapies [34]. Compelling phase I data for the combination of nivolumab with another checkpoint inhibitor, ipilimumab, has led to a phase III front-line trial assessing this combination [35]. These pivotal trials have the potential to drastically change the current treatment paradigm.

23.2.3 Denileukin Diftitox

Several attempts have been made to build upon current immunotherapeutic regimens. The agent denileukin diftitox (DD), approved for the treatment of CD25-positive non-Hodgkin's lymphoma, has been noted to decrease regulatory T-cell (T_{reg}) activity [36]. Given this property, it was thought that DD therapy would augment the activity of IL-2, which has the generalized effect of increasing all T-cell populations (both effector T-cells and T_{reg}s) [37]. A pilot study examined a total of 18 patients with mRCC; a group of 3 patients were initially evaluated for toxicity – the remainder were enrolled after no atypical toxicities were observed [38]. Grade 3/4 toxicities were observed in 11 patients (61 %) receiving high-dose IL-2 and DD, with the most common toxicities including capillary leak syndrome and atrial fibrillation. Of 15 evaluable patients, 5 patients (33 %) demonstrated a response, including three CRs. Peripheral blood analyses did, in fact, reveal substantial reductions in T_{reg}s with DD therapy, declining 56 % from baseline. Further studies are needed to clarify both the efficacy and toxicity of this regimen.

23.2.4 Targeting IL-6

The rationale for targeting IL-6 is multifold; in the context of RCC, elevated levels have been associated with increased metastasis and poor clinical outcome [39]. In addition, increasing levels of IL-6 have been associated with decreasing responsive to therapies such as IL-2 [40]. Rossi et al. reported a phase I/II study of the anti-IL-6 monoclonal antibody, CNTO 328 [41]. Patients had documented mRCC with detectable C-reactive protein (CRP) levels. A total of 11 patients were enrolled in the dose-finding phase I component of the study, and an additional 37 patients were included in the phase II component of the study. In the phase II component, patients were randomized to three schedules of CNTO 328, either 3 mg/kg or 6 mg/kg every 3 weeks for 4 cycles (regimen 1) or every 2 weeks for a total of 6 cycles (regimen 2). The majority of patients enrolled had received prior therapy for mRCC. With respect to efficacy, 1 of 20 patients receiving regimen 1 achieved a PR, while 10 patients (50 %) exhibited SD as a best response. Of the 17 patients receiving regimen 2, no patients achieved an objective response, although 11 patients (65 %) had SD for a median of 80 days. The toxicity profile of CNTO 328 appeared favorable, with no DLTs in the phase I component of the study. There were several serious adverse events (SAEs) recorded, however – one patient receiving regimen 1 suffered from grade 4 cardiac failure after receiving three doses of CNTO 328. Four other SAEs were not ultimately attributed to the antibody. Given the low level of activity seen with CNTO328 in this experience, it is unclear whether further development of the agent is warranted. If pursued, the agent will need to be assessed in combination with other therapies (Table 23.1).

23.3 Angiogenesis Inhibitors: Beyond Direct VEGFR Inhibition

23.3.1 Dual Inhibition of VEGF and MET

There is substantial biological rationale for targeting c-MET signaling in mRCC. Firstly, alterations in *VHL* have been associated with constitutive activation of c-MET in clear cell RCC [42]. Secondly, in the context of papillary RCC,

Table 23.1 Selected emerging immune therapies for mRCC

Agent	Description	Current status/summary of available data
AGS-003	Autologous dendritic cell vaccine	Phase II combination studies with sunitinib reported, with encouraging PFS seen in intermediate- and poor-risk patients. Phase III study underway
IMA901	Vaccine comprised of tumor-associated peptides	Phase II studies reported, with encouraging activity in those patients in whom an immune response is elicited. Phase III study completed; results pending
TG4010	Vaccinia virus expressing IL-2 and MUC-1 antigen	Phase II studies reported, with limited toxicity but no objective response
Nivolumab (BMS-936558)	Monoclonal antibody directed at PD-1	Phase I study included patients with mRCC with encouraging clinical benefit rate and modest toxicity. Phase III assessment underway
Ipilimumab	Monoclonal antibody directed at CTLA4	Phase II data shows higher response rates among patients who incurred immune-related adverse events (i.e., autoimmune hypophysitis, colitis, etc.) Phase III assessment in combination with nivolumab
Tremelimumab	Monoclonal antibody directed at CTLA4	Phase I study in combination with sunitinib therapy shows substantial toxicity
Denileukin diftitox	Diphtheria toxin fragment fused to IL-2	Pilot study in mRCC showed substantial toxicity, but an appreciable response rate (20 % of patients achieved a CR)
CNTO328	Monoclonal antibody directed at IL-6	Phase I/II study showed no objective responses; several serious adverse events were noted

mutations in the tyrosine kinase domain of c-MET are well documented [43]. A phase II trial has been reported which assesses foretinib, a dual inhibitor of c-MET and VEGFR2, in papillary RCC [44]. Patients were divided into two cohorts, receiving the agent at either 240 mg oral daily on days 1–5 of a 14-day cycle or 80 mg oral daily. With a total of 74 patients enrolled, a PFS of 9.3 months was observed with a response rate of 13.5 %. Although the study failed to meet its primary endpoint based on response rate, the PFS in this population compares favorably to that observed with VEGF-TKIs for papillary mRCC. Furthermore, the study provided an opportunity for several key correlatives. Most notably, a total of ten patients were identified with germline MET mutations. Among this cohort, a response rate of 50 % was seen. The study thus points to the potential role of biomarker-based application of this agent in future trials.

A second dual VEGFR2/c-MET inhibitor, cabozantinib, has been assessed in the context of a phase I drug-drug interaction study with rosiglitazone [45]. In contrast to the evaluation of GSK089, this study was limited to patients with clear cell histology. The 25 patients with mRCC enrolled in this experience were heavily pretreated. Most patients had received at least one VEGF-directed therapy (88 %), and over 40 % of patients had received three or more prior therapies. Median PFS in this experience was an impressive 12.9 months, and a response rate of 28 % was observed. These encouraging data have led to a phase III study comparing cabozantinib and everolimus in patients with prior VEGF-directed therapy [46]. The Alliance cooperative group also conducted a randomized phase II study comparing cabozantinib to sunitinib.

A third agent, ARQ 197, specifically antagonizes c-MET. In a phase II study in patients with microphthalmia transcription family (MiT)-associated tumors, three of four patients (75 %) had SD as a best response with ARQ 197 therapy [47]. The agent has been examined in a randomized parallel phase II study (SWOG 1107), in which

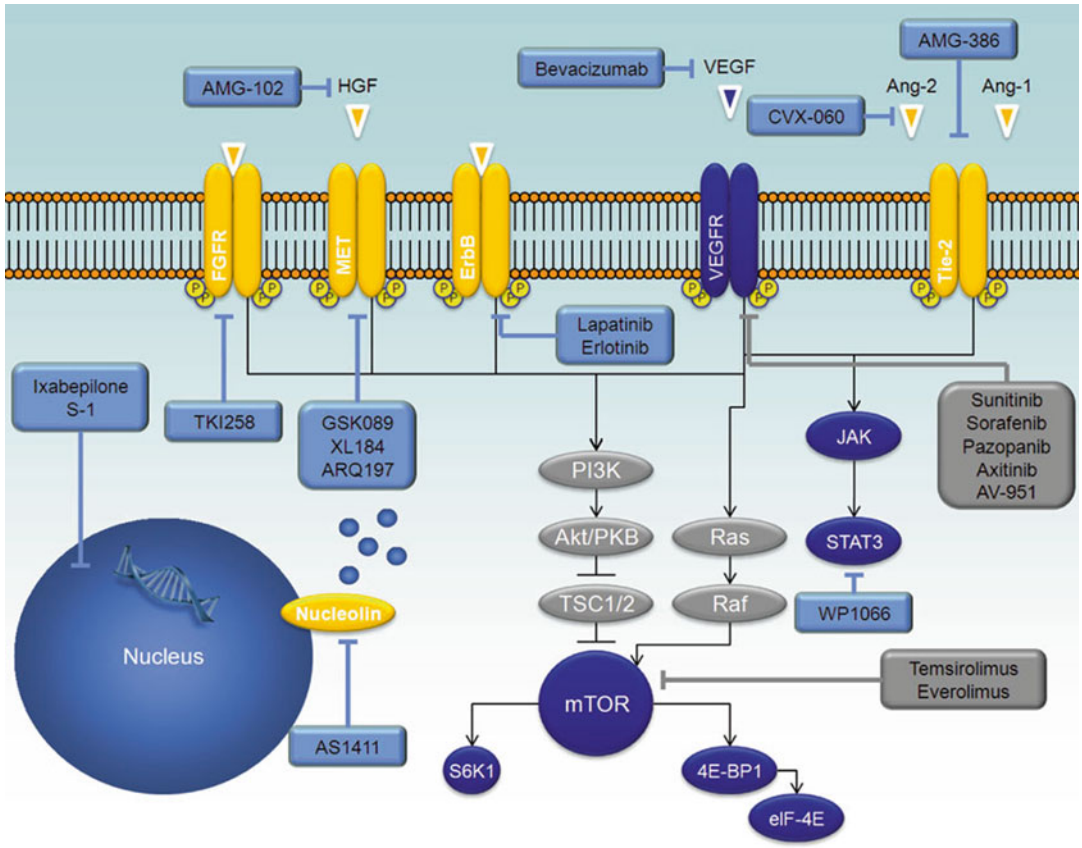

Fig. 23.2 Emerging agents for the treatment of mRCC. Approved agents are denoted in *grey boxes*, while agents currently in clinical development are denoted in *blue boxes*. Note that inhibitors of PI3K/Akt are delineated in other chapters in this textbook

patients with papillary mRCC are treated with either ARQ 197 monotherapy or ARQ 197 in combination with erlotinib. The study is currently closed for an interim analysis.

A second approach to targeting the c-MET signaling axis is depletion of the relevant ligand, hepatocyte growth factor (HGF). Higher levels of this ligand have been associated with a poor prognosis in patients with clear cell RCC [48]. Furthermore, HGF appears to drive tumor growth in those patients with papillary RCC bearing mutations in c-MET [49]. AMG 102 is a humanized monoclonal antibody with affinity for HGF. In a phase II clinical trial, 61 patients with mRCC were treated with AMG 102 at two dose levels, either at 10 mg/kg or 20 mg/kg intravenous every 2 weeks. Patients enrolled had received at least one prior therapy, and although the majority had clear cell disease, 7 patients (11.5 %) had papillary RCC. One PR was observed, and 26 additional patients (43 %) had SD as a best response. Approximately one-third of patients incurred grade 3/4 toxicity, including edema, fatigue, and anorexia. Given the toxicity profile in combination with limited antitumor activity, it is unclear whether further single-agent evaluation of AMG 102 is warranted in mRCC.

23.3.2 Inhibition of Tie-2/Ang-1/2 Signaling

Outside of directly inhibiting VEGF-signaling, other strategies are being devised to inhibit angiogenesis (Fig. 23.2). Recently, attention has been directed to signaling via Tie-2, a cell surface

receptor which promotes pericyte recruitment and maintenance of blood vessel integrity [50]. Two critical ligands have opposing effects on Tie-2 – angiopoietin-1 (Ang-1) activates the receptor, while angiopoietin-2 (Ang-2) inhibits the moiety [50, 51]. Ang-2 is overexpressed in a majority of cancer patients and when present is associated with an aggressive tumor phenotype and poor survival. In the context of RCC, Ang-2 expression is significantly higher in tumor tissue compared to normal renal parenchyma, correlated positively with Tie-2 levels. Furthermore, Ang-2 may be a biomarker of response to antiangiogenic therapy. Bullock et al. compared serum samples derived from 34 patients with mRCC to samples derived from 8 patients with stage I RCC [52]. Ang-2 levels were higher in the former group (median, 3,870 pg/mL *v* 2,489 pg/mL; $P=0.02$). Of the patients with metastatic disease, 26 were evaluated while on therapy with sunitinib. In this group, Ang-2 decreased in 23 patients (88 %). Furthermore, at the time of progression, Ang-2 levels increased in the majority of patients. These preliminary studies provide support for attempts at pharmacologic inhibition of Ang-2. To this end, CVX-060 represents a combination of two peptides with a high affinity for Ang-2. The compound is being evaluated in a phase Ib clinical trial in combination with sunitinib therapy [53]. The combination appears to be well tolerated, and the phase Ib study will serve as a lead-in to a randomized phase II effort comparing sunitinib alone to the combination [54].

While CVX-060 specifically targets Ang-2, there has been some suggestion that dual targeting of Ang-1 and Ang-2 may be a superior strategy [55]. AMG-386 is a peptibody that blocks the interaction of both Ang-1 and Ang-2 with Tie-2 [56]. Preclinical data suggests that VEGF-driven angiogenesis can be mitigated through increasing doses of AMG-386. The agent has been explored extensively in mRCC. A recent, randomized phase II study compared the combination of sorafenib (400 mg oral twice daily) with either one of two dose levels of AMG-386 (3 mg/kg IV weekly or 10 mg/kg weekly) or placebo [57]. Notably, patients who exhibited PD on the placebo arm were offered a continuation of sorafenib with the addition of AMG-386 at 10 mg/kg. The study included patients with clear cell mRCC who had received no prior systemic therapy. The primary endpoint of the study was progression-free survival (PFS).

Ultimately, no significant difference in PFS was observed among patients treated with AMG-386 at 3 mg/kg or 10 mg/kg (8.5 *vs.* 9.0 months, 95 %CI 0.68–1.14; $P=0.523$) [57]. Furthermore, patients receiving placebo had a nearly identical PFS (9.0 months). The confirmed overall RR was higher for patients receiving low- and high-dose AMG-386 (37 % and 38 %, respectively) as compared to placebo (25 %). Toxicity on the experimental arms appeared to parallel that observed on the placebo arm, suggesting that AMG-386 was generally well tolerated and added little to the side effect profile of sorafenib. Although efficacy of AMG-386 was limited in this study, data from other malignancies suggest that doses in excess of 10 mg/kg may yield higher antitumor activity.

While the aforementioned agents specifically target the Ang/Tie signaling axis, regorafenib is an oral TKI that additional binds VEGF receptors and c-kit. This agent has the theoretical advantage of dual pathway inhibition of angiogenesis [58]. Phase I studies demonstrated activity for regorafenib in a number of tumor types including RCC, non-small cell lung cancer, and colorectal cancer with a recommended phase II dose of 160 mg per day for 21 days followed by a 7-day rest [59, 60]. On that basis, a phase II study of 49 evaluable patients given no prior systemic therapy for measurable clear cell predominant advanced or metastatic RCC was conducted [61]. The primary objective was to evaluate the antitumor activity and safety of regorafenib, while secondary objectives included the evaluation of pharmacokinetic and biomarker data [62]. The response rate was 31 % with an additional 50 % experiencing stable disease. Median progression-free survival was 8.2 months with the median overall survival not reached at the time of presentation. Grade 3 or 4 adverse events occurred in 33 (67 %) patients, most commonly hand-foot skin reaction (29 %), renal failure (10 %), and fatigue (8 %). Patients with higher baseline plasma levels of soluble Tie-1 were more likely to have major

tumor shrinkage on therapy. Increased in plasma VEGF-A, VEGF-C, Ang-2, carbonic anhydrase 9, and CK18M30 (a marker of epithelial cell death) and decreased in VEGFR2, soluble Tie-1, and c-kit were seen on therapy. Increased CK18M30 and decreased c-kit were associated with response. Further data from this study are awaited. Regorafenib is being developed in colorectal and non-small cell lung cancer, but a decision on development in RCC is complex given crowding in that market with other VEGF-TKIs.

23.3.3 Thalidomide and Lenalidomide

While the precise mechanism of thalidomide and lenalidomide remains a matter of debate, the agents appear to have both antiangiogenic and immunomodulatory properties akin to other efficacious therapies for mRCC. There have been several attempts to characterize the activity of these agents in mRCC. Choueiri et al. have reported a phase II, open-label study including 28 patients who received lenalidomide at 25 mg oral daily for 3 weeks in a 4 week cycle [63]. Patients had received no more than 1 prior therapy and had a baseline ECOG PS of 0–1. Although no CRs were noted, three patients (11 %) demonstrated a PR and remained progression-free at a follow-up interval exceeding 15 months. Eleven patients (39 %) were noted to had SD >3 months. The median time to treatment failure was 3.7 months, and at the time of publication, median OS had not been reached. Fatigue, skin reactions, and hematologic toxicity constituted the most common grade 3/4 events. A slightly larger trial assessing lenalidomide included 40 patients with mRCC, again limiting entry to patients who had received no more than 1 prior therapy [64]. Among 39 evaluable patients, 4 patients (10 %) achieved an objective response (one CR and three PRs). An additional 20 patients (51 %) had SD lasting ≥6 months. Similarly to the previously noted experience, fatigue and hematologic toxicity constituted the most common adverse reactions. Both of these datasets emerged at roughly the time initial data was presented for the VEGF-TKIs. Although further development of single-agent lenalidomide for mRCC has not been aggressively pursued, there are currently efforts examining the combination of lenalidomide with other targeted agents for mRCC, including sunitinib and everolimus [65, 66].

Several therapeutic trials have also reported the clinical activity of thalidomide therapy in mRCC. Daliani et al. reported an experience including 20 patients with mRCC treated with thalidomide at a starting dose of 200 mg oral daily, with an upward titration to 1,200 mg oral daily as tolerated [67]. Patients had received a median of two prior therapies, primarily consisting of immunotherapy (HD IL-2 or IFN-α). Median TTP was 4.7 months, with a median survival of 18.1 months. Two patients (10.5 %) achieved a PR, and an additional nine patients (50 %) had SD in the range of 3–17 months. A larger experience reported by Escudier et al. assessed 40 patients with advanced disease, with a similar titration to 1,200 mg oral daily [68]. Two patients (5 %) experienced a PR, while nine patients (23 %) had SD lasting greater than 6 months. Significant toxicities were observed in this experience, with three patients experiencing a pulmonary embolism within 12 weeks of treatment initiation and one additional patient experiencing a venous thromboembolism. Neuropathy was observed in 100 % of patients who received thalidomide for a period of 12 months. Ultimately, although corroborating the marginal activity seen with thalidomide in mRCC, this larger experience suggested that the assessed dose could not be recommended due to the extent of toxicity.

Combinations of thalidomide with various agents have been explored. Desai et al. reported a phase II experience assessing the combination of gemcitabine and continuous infusion fluorouracil with thalidomide [69]. Ultimately, it was determined that thalidomide added little to the efficacy of the cytotoxic regimen but added substantial vascular toxicity. Combinations of thalidomide with immunotherapy have also been attempted; Hernberg et al. reported a phase II clinical trial evaluating the combination of IFN-α and thalidomide [70]. Although the regimen assessed

appeared to be feasible, thalidomide added little to the anticipated clinical benefit from IFN-α alone. Thalidomide therapy has also been assessed in the adjuvant setting, with somewhat sobering results. Patients with high-grade T2 disease, T3/T4 disease, or nodal positivity were randomized to receive either thalidomide 300 mg oral daily for 24 months or observation. After enrollment of a total of 46 patients, there was an inferior 2-year recurrence-free survival (RFS) observed on the thalidomide arm (47.8 % *v* 69.3 %, $P=0.022$).

23.3.4 Thrombospondin-1 Agonism

Activated by *p53*, thrombospondin-1 inhibits the activity of VEGF and basic fibroblast growth factor (bFGF), both putative mediators of angiogenesis [71, 72]. A phase II study examined two dose levels of the thrombospondin-1 analogue, ABT-510, in patients with treatment-naïve mRCC [73]. With a total of 103 patients enrolled, 51 patients were randomized to a dose of 10 mg subcutaneously twice daily, while 52 were randomized to receive 100 mg subcutaneously twice daily. The majority of patients in this study had clear cell disease (76 %) and had a baseline ECOG PS of 0 (70 %). There were no differences in PFS or RR between patients receiving 10 and 100 mg doses of ABT-510 (PFS: 4.2 *vs.* 3.3 months, respectively, $P=0.803$; RR: 4 % *v* 0 %, respectively; $P=0.243$). Although the agent had limited toxicity (a total of 4 grade 3/4 events were noted), the efficacy observed in this study was not thought to justify further investigation of the single agent.

23.4 Other Novel Targets in mRCC

23.4.1 Targeting Fibroblast Growth Factor Receptor (FGFR)

FGFR signaling is a putative escape mechanism for cancer cells exposed to VEGF-directed therapies [74]. Although the small-molecule dovitinib has affinity for the VEGF family of receptors and other receptor tyrosine kinases, it uniquely binds FGFR1-3 with high affinity [75]. A phase I/II study has explored the activity of dovitinib therapy in mRCC patients refractory to standard treatment [76]. The phase I component of the study was recently reported, including 20 patients that had received a range of prior therapies, including VEGF-TKIs (80 %), mTOR inhibitors (55 %), and the immunotherapy (15 %). Confirmed PRs were observed in two patients (10 %), and seven patients (35 %) achieved SD as a best response. Notably, in the subset of ten patients who had received both VEGF-TKIs and mTOR inhibitors, one patient exhibited a PR and six patients had SD as a best response.

Based on these encouraging preliminary results in a heavily refractory population, a phase III trial was performed to compare dovitinib to sorafenib as a third-line therapy in patients with mRCC that had received one VEGF-TKI and one mTOR inhibitor [77]. The study accrued a total of 570 patients and ultimately failed to meet its primary endpoint of improvement in PFS. PFS associated with dovitinib was 3.7 months, as compared to 3.6 months with sorafenib ($P=0.063$). Although the study was negative, it does provide some insights into future benchmarks for clinical trials in mRCC done in the third-line setting.

23.4.2 ErbB Targeting

Several attempts have been made to assess the role of ErbB-directed therapies in mRCC. Preclinical studies in RCC-derived cell lines suggested that the presence of wild-type *VHL* was associated with increased responsiveness to the EGFR-directed monoclonal antibody C225 [78]. On the basis of these data, Southwest Oncology Group (SWOG) trial 0317 assessed the EGFR tyrosine kinase inhibitor erlotinib in patients with papillary renal cell carcinoma [79]. Patients in this study had not received prior chemotherapy or immunotherapy and were treated with erlotinib at 150 mg oral daily until the time of disease progression. Of 45 evaluable patients, 5 patients (11 %) achieved a response to therapy, with 24 additional patients achieving stable disease. The median OS in this population was 27 months. Although the study failed to meet the prespecified

endpoints for response rate, the clinical benefit ascribed to erlotinib therapy was deemed to be encouraging. Several subsequent efforts have examined other combinations with erlotinib. Flaig et al. reported a study assessing erlotinib with sirolimus in patients with metastatic RCC (albeit not restricted to clear cell disease) [80]. Patients in this study had previously progressed on therapy with sunitinib or sorafenib therapy. No responses were observed to this regimen, and median PFS was 12 weeks. These data failed to support further exploration of this regimen as an alternative to other available second-line therapies. Combination therapy has also been assessed in the context of the treatment-naïve patient – a randomized phase II study comparing bevacizumab with or without erlotinib showed no difference in RR (14 % with the combination *v* 13 % with bevacizumab alone), and no benefit in PFS (9.9 months with the combination *v* 8.5 months with bevacizumab alone, $P=0.58$) [81]. A separate regimen of bevacizumab, imatinib and erlotinib has also been explored in a phase I/II study; this regimen yielded unacceptable toxicity (grade 3/4 diarrhea, rash and fatigue) [82].

Outside of EGFR, other moieties in the ErbB family have been assessed as therapeutic targets in mRCC. As one notable example, a phase III clinical trial was conducted using the dual-targeting small-molecule inhibitor lapatinib, which antagonizes both EGFR and HER2. In this study, 416 patients with mRCC were randomized to receive either lapatinib or hormonal therapy (tamoxifen or medroxyprogesterone). Patients were eligible if any level of immunohistochemical staining for HER2 (1+, 2+ or 3+) was observed and if they had progressed on prior cytokine-based therapy. Median TTP was 15.3 weeks with lapatinib as compared to 15.4 weeks with hormonal therapy ($P=0.60$). OS was also comparable between lapatinib and hormonal therapy (46.9 *vs.* 43.1 weeks, respectively; $P=0.29$). In the subset of 241 patients with 3+ staining, there was a more appreciable difference in clinical outcome – there was a trend toward improvement in TTP with lapatinib therapy (15.1 *vs.* 10.9 weeks, $P=0.06$) and a significant improvement in OS (46.0 *vs.* 37.9 weeks; $P=0.02$).

23.4.3 Targeting Nucleolin

Oligonucleotide aptamers represent short nucleic acid sequences that exhibit conformal binding to proteins. The novel aptamer AS1411 represents one such molecule that specifically targets nucleolin. Nucleolin is a protein with multiple purported roles and is found predominantly in rapidly dividing cells [83]. It is presumed to function in ribosome production and chromatin organization in the nucleolus [84]. Further, it may serve as a cell surface receptor for a variety of ligand growth factors [85]. Preclinical data suggested antitumor activity of AS1411 in the DU145 prostate cancer cell line, stimulating further clinical development of this agent [86].

A phase II, single-arm trial was conducted to evaluate the efficacy of AS1411 in mRCC [87]. The agent was administered to patients with clear cell histology who had failed one or more prior therapies at a dose for 40 mg/kg/day for days 1–4 of a 28-day cycle. Patients received only 2 cycles of therapy. With 35 patients enrolled, 1 patient exhibited a PR, and 21 patients (60 %) had SD as a best response. No grade 4 or 5 toxicities were observed; the most common adverse effects were diarrhea and fatigue. It remains to be seen whether further combination studies of the drug will be pursued, given both the modest toxicity and efficacy of the agent (Table 23.2).

23.5 Cytotoxic Chemotherapy

23.5.1 S-1

Although cytotoxics have been largely displaced by targeted therapies and immunotherapies for mRCC, there have been several recent evaluations of novel cytotoxic agents. Naito et al. reported an experience evaluating the novel fluorinated pyrimidine S-1 [88]. S-1 represents a composite of tegafur, potassium oxonate, and 5-chloro-2,4-dihydroxypyrimidine in an oral formation. In a multicenter phase II trial, 45 patients with prior cytokine therapy or a contraindication to cytokine therapy were enrolled. The majority of patients in this experience had received IFN-α, IL-2,

Table 23.2 Selected emerging agents for mRCC that inhibit novel angiogenic signaling axes

Agent	Description	Current status/summary of available data
CVX-060	Monoclonal antibody fused to 2 peptides with high affinity for Ang-2	Phase Ib/II combination study with sunitinib ongoing
AMG-386	Peptibody that blocks the interaction of Ang-1/2 with Tie-2	Phase II study comparing sorafenib with placebo or AMG-386 (at 2 dose levels) showed no improvement in PFS with the addition of AMG-386
Regorafenib	TKI with affinity for Tie-2, VEGFR2, and c-kit	Phase II study shows promising RR and PFS
Thalidomide	Antiangiogenic and immunomodulatory agent	Phase II data for single-agent therapy shows modest clinical benefit with substantial toxicity. Combinations with immunotherapy and cytotoxic agents show little synergy but added toxicity. Adjuvant data from small series discouraging
Lenalidomide	Antiangiogenic and immunomodulatory agent	Phase II studies with differing reports of clinical benefit; combination studies with sunitinib and everolimus ongoing
ABT-510	Thrombospondin-1 analogue	Phase II study with minimal response

or both; a small fraction (<15 %) had received prior therapy with a VEGF-directed agent (either sunitinib or sorafenib). Eleven patients (24.4 %) had a PR, while 28 additional patients (62.2 %) had SD as a best response. Median PFS was 9.2 months, and with a median follow-up of 21.7 months, median OS had not been reached. The toxicity profile of S-1 was manageable, with the most common grade 3/4 events being neutropenia and anorexia. Accompanying correlative studies showed that expression of thymidylate synthetase (TS) mRNA was lower in responders ($P=0.048$) and that below median levels of TS mRNA expression were associated with a longer PFS ($P=0.006$).

23.5.2 Ixabepilone

Ixabepilone has been assessed in the context of two phase II studies. Posadas et al. reported one such trial with an initial planned accrual of 37 patients [89]. Patients bearing any RCC histology were eligible, and any number of prior therapies was permitted. Ixabepilone was administered at a dose of 40 mg/m^2 every 3 weeks until progression. No responses were observed among the first 12 patients enrolled, thereby sufficing the stopping rules for the study. Of these patients, six achieved SD as a best response. Toxicities encountered were akin to those seen in studies of ixabepilone in breast cancer – the most common grade 3/4 events were lymphopenia, neutropenia, leucopenia, diarrhea, and infection.

Huang et al. evaluated a different dose and schedule of ixabepilone in a larger cohort of patients [90]. In this study, ixabepilone was administered at 6 mg/m^2 for 5 days every 3 weeks. Of 87 patients enrolled, over half (52 %) had received no prior systemic therapy. The remainder of patients were principally treated with immunotherapy. The ORR in this study was 12.6 %, with one CR and ten PRs. A further 33 patients (37.9 %) had SD as a best response. The most common treatment-related adverse events were alopecia, gastrointestinal toxicity, and fatigue. The study was paired with a number of correlative efforts, one of which included biopsies at baseline and after five treatments with ixabepilone. Supporting the mechanism of this agent, microtubule targeting was demonstrated in 85–90 % of patients. In further explorations of *VHL* mutational status relative to clinical response, no correlation was observed.

Other strategies to target microtubule dynamics have also been attempted in mRCC. The agent

ispinesib (SB-715992) targets the mitotic kinesin spindle protein, triggering cell cycle arrest [91, 92]. A phase II trial conducted by the University of Chicago Consortium included 20 patients with mRCC who had received between one and two therapies within 8 months of enrollment. Patients were treated with ispinesib at a dose of 7 mg/m^2 intravenously on days 1, 8, and 15 of a 28-day cycle. The majority of patients had clear cell histology. Of 19 evaluable patients, no objective responses were observed. Only six patients had SD after 8 weeks of therapy. Although limited grade 3/4 toxicities were observed, the rather dismal efficacy of ispinesib in this experience suggests that the utilized dose and schedule should not be carried further.

23.6 Future Directions

Although VEGF- and mTOR-directed therapies have vastly altered the current treatment paradigm for mRCC, the fact remains that the disease remains incurable. In the coming years, the research community will be prompted to look toward novel therapies that target distinct pathways and employ unique mechanisms. The focus of the current chapter is principally on agents that have shown a signal of activity in mRCC in published reports. However, the pipeline of potential therapies extends far beyond those discussed herein. Many of these therapies may be "borrowed" from other disease states, based on commonalities observed with RCC. For instance, rearrangements in *ALK* have recently been noted in the context of pediatric variants of RCC [93, 94]. The agent crizotinib, which shows promise in non-small cell lung cancer patients bearing *ALK* rearrangements, may thusly be investigated in a subset of patients with mRCC [95, 96]. Ultimately, an understanding of the biology of renal cell carcinoma will hopefully drive therapeutic selection. With data emerging from comprehensive efforts such as The Cancer Genome Atlas (TCGA) project, this biology will be better characterized in the years to come.

Clinical Vignette

A 68-year-old male was diagnosed with de novo metastatic renal cell carcinoma several years ago after presenting to his primary care physician with symptoms of shortness of breath. Initial imaging studies showed multiple 1–2 cm pulmonary nodules. Full staging thereafter (including computerized tomography of the abdomen and pelvis and bone scan) showed no bone metastasis, but a 6 cm left lower pole renal mass. Jim received a partial nephrectomy showing clear cell RCC, Fuhrman grade 2/4. Biopsy of the pulmonary lesion revealed clear cell carcinoma consistent with a renal primary. He received initial therapy with high-dose interleukin-2 (IL-2). Although he tolerated the regimen well, he had radiographic progression within 2 months of completing treatment. He was then initiated on therapy with sunitinib but progressed after approximately 9 months of therapy. He received the mTOR inhibitor everolimus next but had early stigmata of interstitial lung disease after just 2 months and was thus discontinued therapy. Thereafter, he progressed on further sequential therapy with sorafenib and pazopanib. Despite exposure to five prior lines of therapy, Jim maintains an excellent performance status. His oncologist is appropriately seeking clinical trials exploring novel therapies for this disease.

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