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**LIMITATIONS OF SELECTION CRITERIA OF ONGOING RANDOMIZED  
CONTROLLED TRIALS TESTING TARGETED THERAPIES IN AN  
ADJUVANT SETTING OF PATIENTS WITH RENAL CELL CARCINOMA**

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# Abstract

**Introduction and objectives:** 40-50% of patients who underwent partial or radical nephrectomy for localized clear cell renal cell carcinoma (CCRCC) develop metastases during the follow-up period. Until now adjuvant chemotherapy and/or adjuvant immunotherapy failed to demonstrate significant advantages to reduce the risk of progression in CCRCC. Recently, new targeted therapies such as tyrosine kinase or mTOR inhibitors showed good results in the treatment of metastatic diseases. Therefore, the use of this new category of drugs was tested also in an adjuvant setting of high-risk patients to prolong the progression-free survival after partial nephrectomy (PN) or radical nephrectomy (RN). Indeed, 5 randomized control trials (ASSURE, STRAC, SORCE, EVEREST, PROTECT) are currently ongoing with the aim to test the role of some of these drugs (Sunitib, Sorafenib, Everolimus, Pazopanib) in comparison with placebo. The wide variability of the inclusion criteria used to enroll patients can affect the sample size and consequently the number of expected events according to the planned primary endpoint. In our study we tested the appropriateness of the eligibility criteria used to recruit participants for clinical trials on adjuvant medical therapy and we tested whether the simultaneous presence of loss of chromosomes 9p and 14q was associated with a different risk of recurrence in the subgroup of patients suitable for adjuvant therapy with targeted therapies.

**Materials and methods:** Clinical records of 5,463 patients with RCC who received PN or RN were gathered from the databases of 16 Italian Urology Clinics. We selected all non-metastatic cases. Moreover, all patients who underwent cytoreductive nephrectomy for metastatic disease and those with non-clear cell RCC were excluded. The following pathological variables were assessed: local extension and dimension of primary tumor, regional lymph nodes involvement, Fuhrman's nuclear grade and coagulative necrosis. All the included cases were reclassified according the eligibility criteria of each ongoing RCT testing adjuvant targeted therapies. For each category we calculated the frequencies and the progression-free survival. Events were defined as patients who developed distant metastases during the follow-up period.

Finally, we evaluated the loss of chromosomes 9p and 14q in 175 patients who underwent PN or RN for non-metastatic ccRCC. We generated different multivariable models with the intent of demonstrating the independent predictive role of cytogenetic abnormalities once adjusted for the effects of the most common tools used to stratify patients in ongoing phase 3 trials evaluating the efficacy of adjuvant therapies.

**Results:** The most selective criteria were used in the context of STRAC study. Indeed, using the high risk and very high risk categories according to UISS system, only 12% of our patients resulted suitable for randomization. At a median follow-up of 60 months, the percentage of observed events were 36% for high-risk and 68% for very high risk category, respectively. Conversely, the less selective criteria were used in ASSURE, EVEREST and SORCE trials in which the enrolled patients ranged between 41-43% of cases. In particular, the inclusion of patients in pT1b G3-4 or pT2 categories regardless the Fuhrman grading resulted in a very limited number of events ranging between 13-21% of cases. Similarly, the inclusion of intermediate risk patients according to Leibovich criteria in the SORCE trial is associated with only 20% of events.

Concerning the cytogenetic analyses performed in a small subgroup of cases, no cytogenetic abnormalities were observed in 135 cases (77.1%), and loss of chromosome 9p or 14q was detected in 14 cases (8%) and 9 cases (5.1%), respectively. The contemporary presence of both cytogenetic alterations was reported in 17 cases (9.7%). The median follow-up duration was 36 mo (interquartile range: 21–78). The simultaneous loss of both chromosomes 9p and 14q turned out to be an independent predictor of DFS, once adjusted for the effects of pT and nuclear grade (hazard ratio [HR]: 4.579; 95% confidence interval [CI], 1.767–11.868), Leibovich score (HR: 3.704; 95% CI, 1.565–8.768), or UCLA Integrated Staging System (UISS; HR: 3.194; 95% CI, 1.351–7.553).

**Conclusions:** Ongoing RCTs testing the adjuvant effect of targeted therapies in patient who underwent radical or partial nephrectomy for non-metastatic RCC were strongly limited by the used selection criteria. Indeed, in the majority of these trials the researchers enrolled categories with a very limited risk to progress influencing significantly the number of events needed to demonstrated a statistically significant differences between treatment arms and placebo ones.

Moreover, loss of chromosomes 9p and 14q was an independent predictor of DFS in patients who underwent PN or RN for nonmetastatic ccRCC, once adjusted for the effects of either Leibovich score or UISS, demonstrating that the recurrence-free survival of patients suitable for adjuvant protocols could be strongly influenced by the cytogenetic characteristics of the tumor.

## **Riassunto**

Introduzione ed obiettivi: Il 40-50% dei pazienti con carcinoma renale parenchimale a cellule chiare localizzato sviluppa metastasi a distanza nel corso del follow-up dopo nefrectomia parziale o radicale. Al momento attuale, nessun trattamento chemio e/o immunoterapico adiuvante si è dimostrato efficace nel ridurre o dilazionare il rischio di progressione nei pazienti con carcinoma renale parenchimale a cellule chiare. I risultati favorevoli riportati negli ultimi anni con l'impiego degli inibitori delle tirosin-chinasi o di mTOR nel trattamento del carcinoma renali a cellule chiare metastatico ha lasciato ipotizzare anche un loro utilizzo adiuvante nei pazienti con maggiore rischio di progressione di malattia dopo nefrectomia parziale o radicale. Sono attualmente in fase di svolgimento 5 studi clinici randomizzati di fase III (ASSURE, STRAC, SORCE, EVEREST, PROTECT) che confrontano alcuni di questi farmaci (Sunitinib, Sorafenib, Everolimus, Pazopanib) al placebo. In questi studi di fase III sono stati utilizzati criteri di selezione estremamente variabili con evidenti conseguenze sul campione statistico necessario e sul numero di eventi attesi per il raggiungimento dell'obiettivo primario dello studio. Obiettivo della ricerca sarà quello di verificare l'appropriatezza dei diversi criteri utilizzati per selezionare i pazienti da arruolare nei diversi trials di adiuvante.

Materiali e metodi: L'analisi è stata effettuata su un database multicentrico di 5463 pazienti sottoposti a nefrectomia radicale (NR) o nefrectomia parziale (NP) per carcinoma renale parenchimale in 16 centri italiani di urologia. Sono stati selezionati tutti i pazienti sottoposti a NR o NP per carcinoma renale non metastatico a cellule chiare. Sono stati esclusi dall'analisi tutti i pazienti sottoposti a nefrectomia citoreduttiva per malattia metastatica e tutti i pazienti con istotipo neoplastico non a cellule chiare. Per ogni singolo paziente sono state valutate le seguenti variabili cliniche e patologiche: stadio patologico del tumore primitivo, stadio patologico dei linfonodi regionali, grading nucleare secondo Fuhrman, performance status ECOG, dimensioni del tumore primitivo e necrosi coagulativa. La valutazione delle suddette variabili ha consentito di riclassificare tutti i casi inclusi in analisi in accordo con i criteri di selezione identificati nei 5 RCTs attualmente in corso di realizzazione. Utilizzando i dati retrospettivi del database SATURN abbiamo calcolato e confrontato il numero di casi arruolabili in accordo con i criteri di inclusione riportati nei differenti trials di terapia adiuvante attualmente in corso. Inoltre, abbiamo calcolato il numero di eventi (pazienti in progressione di malattia) e la relativa sopravvivenza libera da progressione dei diversi sottogruppi suscettibili di arruolamento nei diversi trials.

### Analisi correlate

In un sottogruppo di questi pazienti abbiamo eseguito la revisione centralizzata dei preparati istologici con l'obiettivo di valutare il ruolo prognostico addizionale di alcuni marcatori citogenetici testati con l'utilizzo della metodica FISH.

# RENAL CELL CARCINOMA

## Introduction

Renal cell carcinoma (RCC) accounts for 3% of all adult malignancies and is steadily increasing at a rate of about 2.5% per year across population groups with the greatest increase observed in the incidence of localized tumors [1-3]. RCC is a highly aggressive tumor and the most lethal of urologic malignancies with more than 40% of patients dying from their cancer [4]. Approximately one-third of patients present with metastases and up to 40% of patients undergoing nephrectomy for clinically localized RCC will develop local recurrence or metastatic disease [5]. Significant advances in the diagnosis, staging, and treatment of patients with RCC during the last 2 decades have resulted in improved survival of a select group of patients and an overall change in the natural history of the disease [2,3,6]. Current research efforts are focused on understanding the genetic and protein expression profiles of the various histologic subtypes of RCC. Molecular profiling of individual tumors will likely improve treatment outcome and guide patient selection for targeted therapies.

Currently, tumor stage at diagnosis remains the most important prognostic factor: the probability of survival at 5 years, amounting to 80 to 100% in patients with localized disease at diagnosis, is reduced up to 12% for those with distant metastases, for which the median survival does not reach the year. Consequently the natural history, the symptomatic RCC is difficult in the early stages, while about 20-25% of patients present advanced disease already at diagnosis and in less than 5% of these is found only one metastatic site. [7] The main prognostic factors able to predict the risk of recurrence and/or distant progression and survival of patients with RCC can be classified into anatomical, histological, molecular, cytogenetic, clinical and laboratory [8].

To increase the prognostic accuracy, during the last years a series of mathematical models has been created founded on the integration of the prognostic information furnished by the single clinical and pathological variables. Some of these prognostic systems are proposed as important tools for the planning of the therapy and the rationalization of the follow-up of the patients on the base of the different risk of progression. [9,10]

Some integrated prognostic systems have recently been used to define the criteria for inclusion and stratification of patients with RCC enrolled in randomized clinical trials evaluating the efficacy of adjuvant medical therapy after surgery of the primary tumor [10,11].

## 1 Anatomical prognostic factors

Anatomic criteria have been used to traditionally stage RCC and have historically served as the best prognostic tools. The first to propose a formal staging system stratified patients according to the involvement of the tumor according to the physical characteristics of the tumor and the position of the spread of the tumor were Flocks and Kadesky in 1958 [12].

The anatomical prognostic factors are represented by the dimensions of the neoplasia, extension of the primitive tumor, the vascular invasion, the invasion of the renal capsule, the involvement of the adrenal gland, the involvement of the place-regional lymph nodes and the presence of distance metastasis. The collection of this information is covered in Tumour Nodes Metastasis (TNM) classification, a dynamic system of staging which is periodically updated based on principal evidences coming from the literature. There are many evidences in the literature regarding the prognostic importance of the size of the primary tumor. Some Authors have indicated as tumors larger than 10 cm may be more aggressive than those of size between 7 and 10 cm [13].

The TNM system has undergone systematic revisions to mirror the improved results attained in the management of RCC. Although the TNM staging system provides good prognostic information, it continues to be scrutinized as a result of contradictory emerging data.

### 1.1 Tumor size

Primary tumor size is a key component of the TNM staging system and remains one of the most important prognostic factors for RCC [14,15]. Studies have demonstrated that survival varies in relation to tumor size with 5-year survival rates of 84%, 50%, and 0% for patients with tumors measuring <5 cm, 5–10 cm, and >10 cm, respectively [16]. In 1997, the cut-off size for T1 stage was expanded from 2.5 to 7 cm, which has led to controversy [17], [18]. Multiple studies have evaluated the optimal T1 size criteria for patients undergoing either partial or radical nephrectomy suggesting alternative cut-offs, which include 4.5 cm [19], 5 cm [20], 5.5 cm [21], 8 cm [22], and 10 cm [23]. Although these studies disagree about the optimal cut-off, they all demonstrate that primary tumor size is an important determinant of prognosis. The increasing widespread and now mainstream use of partial nephrectomy for smaller tumors has also made the T1 cut-off criteria not only important in terms of prognostic value, but also in relation to eligibility for partial

nephrectomy. Hafez et al. [24] attempted to delineate the optimal cut-off size for tumors amenable to partial nephrectomy. In this study, patients with T1 tumors  $\leq 4$  cm who underwent partial nephrectomy had significantly better survival compared to those with larger tumors. Over the past decade, evidence from other major clinical series has shown the effectiveness and safety of partial nephrectomy in the treatment of renal tumors  $\leq 4$  cm [25], [26], [27], [28], [29], [30]. As a result, the 2002 TNM T1 category was amended to T1a and T1b, based on a 4 cm cut-off [31]. Although elective partial nephrectomy is generally performed in patients with tumor less than 4 cm, there is emerging data that it can be performed on patients with larger tumors that are anatomically amenable, provided an adequate surgical margin can be safely obtained [32-35].

Several investigators have attempted to further improve prognostic accuracy of T2 tumors by stratifying based on size. Frank et al. [36] analyzed 544 patients with T2 tumors and found that tumors  $>10$  cm behaved more aggressively compared to those between 7 and 10 cm after adjusting for regional lymph node involvement and distant metastases. A recent international multicenter study consisting of 706 patients with stage T2 RCC demonstrated that tumors  $>11$  cm were associated with presence of metastatic disease compared to those between 7 and 11 cm [37]. Stratification by tumor size cut-off of 11 cm demonstrated 5- and 10-year survival rates of 73% and 65% for T2 tumors  $\leq 11$  cm, and 57% and 49% for T2  $>11$  cm, respectively. Furthermore, tumor size was retained as an independent prognostic factor for survival; and as the strongest prognostic factor for patients with T2N0M0 disease. These data suggest that a T2a and T2b subclassification to reflect these findings may improve identification of patients at high risk for disease progression and eligibility for adjuvant clinical trials.

## 1.2. Perinephric/renal sinus fat involvement

The 5-year Disease-specific Survival (DSS) for stage T3 disease ranges from 37 to 67%, which reflects this broad category that includes various clinical situations that involve tumor extension beyond the renal capsule [38,39]. Tumors that extend into the perirenal fat, but not beyond Gerota's fascia or have direct adrenal involvement are currently classified as T3a. Perinephric and renal sinus fat invasion are both currently classified as T3a RCC. The impact of fat invasion on prognosis of patients with RCC is well documented [39-41]. However, different locations of fat invasion have been reported to portend different prognoses. Thompson et al. [42] examined 162 patients with peripheral perinephric fat invasion and 43 with renal sinus fat invasion, respectively. Patients with renal sinus fat invasion had a higher risk of regional lymph node metastases, higher grade, and a greater incidence of sarcomatoid differentiation. Patients with renal sinus fat invasion were 1.6 times more likely to die of RCC compared to those with perinephric fat invasion [42]. Furthermore,

the risk of death persisted in multivariate analysis after adjusting for regional lymph node involvement and presence of distant metastases. The presence of renal fat involvement also appears to increase the risk of death from RCC among patients with venous tumor thrombus [40,43]. Leibovich et al. [40] reported that among 422 patients with stage T3b disease, those with concomitant perinephric or renal sinus fat invasion were 1.87 times more likely to die of RCC compared to patients without fat invasion.

The role of tumor size in T3a tumors has attracted little attention in the literature. Siemer et al. [44] analyzed 237 patients with perinephric fat invasion and identified an ideal tumor size cut-off of 7 cm. Patients with T3a tumors  $\leq 7$  cm yielded similar survival to patients with T1 tumors and patients with T3a tumors  $>7$  cm yielded similar survival to T2 tumors. The current T1-2N0M0 and T3aN0M0 classification for RCC has been debated in other studies. Murphy et al. [45] reported that worse disease-free survival (DFS) and DSS for patients with T2N0M0 disease compared to those T3aN0M0 disease suggesting that tumor size was a stronger prognostic factor than tumor invasion through the renal capsule. Gilbert et al. [46] also showed that 5-year DFS rates were  $>90\%$  for patients with either T1-2N0M0 or T3aN0M0 disease. Siddiqui et al. [47] investigated the association of perinephric and renal sinus fat invasion with death from RCC independent of tumor size. Patients with T3a disease were subdivided into three groups according to tumor size to match the size definitions for the T1a, T1b and T2 tumor classifications. Patients with fat invasion and tumors  $\leq 4$  cm, 4–7 cm and  $>7$  cm were 6.15, 4.13, and 2.12 times, respectively, more likely to die from RCC compared to those without fat invasion. These associations remained statistically significant in a multivariate analysis that included nuclear grade and histological coagulative tumor necrosis independent of tumor size.

Lam et al. [48] recently reported on an international multicenter study of 623 T3a tumors with fat involvement only. There was no difference in DSS between patients with T2N0M0 and T3aN0M0 tumors  $\leq 7$  cm, but both groups were superior to patients with T3aN0M0 tumors  $>7$  cm. Furthermore, multivariate analysis demonstrated that patients with T3a tumors  $>7$  cm had a 1.36-fold increased risk of death from RCC compared to patients with T3a tumors  $\leq 7$  cm and T2 tumors. In addition, patients with T3a tumors  $>7$  cm had the same prognosis as patients with T3b tumors. These findings suggest that patients with non-metastatic T3a tumors  $>7$  cm are at higher risk for death from RCC and should be considered for future clinical trials investigating adjuvant therapies.

### 1.3. Adrenal gland invasion

Few patients present with RCC involving the ipsilateral adrenal gland at the time of diagnosis [49, 50]. The current TNM staging system categorizes patients with adrenal involvement into the T3a



group, which also includes tumors that extend into perirenal fat, but not beyond Gerota's fascia. Recent reports have shown that patients with direct extension into the adrenal gland fair worse than those with extension into perirenal fat only [51,52]. Han et al. [51] first reported that among patients with adrenal involvement, the median survival was 12.5 months and the 5-year DSS was 0%, whereas the median survival was 36 months and the 5-year DSS was 36% for those with perinephric fat invasion and no adrenal involvement. Furthermore, the survival of patients with T3a disease and adrenal involvement was not better than patients with T4 tumors. Although a correlation existed between adrenal invasion and higher tumor grade, lymph node involvement, and metastatic disease, multivariate analysis demonstrated that adrenal invasion was an independent predictor of poor prognosis.

In addition, it has been shown that stage for stage, patients with direct adrenal invasion fair worse than those without [53,54]. Others have corroborated the above findings concluding that tumors with adrenal involvement from direct extension appear to have a similar outcome to patients with T4 disease [52,55]. Paul et al. [56] evaluated the outcome of patients with adrenal metastasis in renal tumors reporting a mean survival of 15 months in 21 patients with adrenal gland involvement from hematogenous spread compared to 84 months in 339 patients without adrenal gland involvement. It is important to differentiate between an upper pole tumor that directly invades the adrenal gland from that of a metastatic deposit. Cases of metastatic involvement of the adrenal gland should be classified as M1 and have a poor prognosis. However, no studies to date have directly evaluated the prognostic significance of ipsilateral adrenal involvement due to hematogenous spread versus direct extension.

Several studies have suggested that removal of the ipsilateral adrenal gland is not routinely necessary during radical nephrectomy [49,57,58]. The low local recurrence rate following partial nephrectomy for RCC, in which by definition, adrenalectomy is not performed, further questions the value of routine adrenalectomy [28]. Paul et al. [57] examined 866 patients who underwent radical nephrectomy plus ipsilateral adrenalectomy for RCC. Twenty-seven (3.1%) patients had adrenal metastasis, of whom 4 had bilateral or contralateral adrenal involvement. In this study, tumor size >8 cm and M stage were independent predictors of adrenal involvement. Current evidence suggests that the rate of adrenal metastasis is low and that modern day imaging modalities are sensitive enough to pick up adrenal lesions. These findings were confirmed in a contemporary prospective series of 511 nephrectomies, in which computed tomography (CT) was greater than 99% specific and nearly 90% sensitive to detect adrenal involvement preoperatively [50]. Given the low percentage of patients with adrenal involvement and use of detailed preoperative imaging, the vast majority of those with RCC can be spared the potential morbidity associated with ipsilateral

adrenalectomy. Siemer et al. [58] compared 1010 patients that underwent radical nephrectomy with simultaneous ipsilateral adrenalectomy for RCC to 625 patients that did not have simultaneous adrenalectomy. Adrenal gland metastases were found in 56 of 1010 patients (5.5%) undergoing nephrectomy plus adrenalectomy. Of 30 patients with adrenal metastasis and a preoperative CT/magnetic resonance imaging (MRI), 23 were found to have histological evidence of cancer, approaching a false-negative rate of 23.3%. All patients with false-negative CT/MRI had a primary tumor of >4 cm and patients with adrenal metastases predominately had stage T3 or greater (82%). However, there was no significant difference in DSS (75% vs. 73%) or postoperative complications (7% vs. 8%) between patients that underwent adrenalectomy compared to those that did not. A final caveat to consider regarding routine adrenalectomy for all patients with RCC is the possibility of developing a future, contralateral adrenal metastasis. If these patients become functionally or surgically adrenal insufficient and require steroid replacement, this could negatively impact on their eligibility to receive systemic immunotherapy.

#### 1.4. Venous tumor thrombus extension

RCC invades the venous system in 4–9% of newly diagnosed patients [59,60]. In 1997, inferior vena cava (IVC) tumor thrombus located above the diaphragm, previously stage T4, was changed to T3c, and thrombus involvement below the diaphragm, previously staged T3c, was changed to T3b with renal vein (RV) involvement [61]. Most studies have found no difference in survival based on the level of IVC involvement [62,63] or based on the involvement of RV versus IVC [64]. However, it has been suggested recently that long-term survival may be significantly better in patients with RV involvement compared to IVC involvement. Moinzadeh and Libertino [65] reviewed 153 patients that underwent nephrectomy and tumor thrombectomy and concluded that long-term survival of patients with RV involvement was significantly greater than those with IVC involvement. Kim et al. [66] compared 226 patients who underwent nephrectomy and RV or IVC tumor thrombectomy with 654 patients that underwent nephrectomy without venous involvement. In patients with localized RCC (N0M0), DSS was similar in patients with RV (T3b) and IVC involvement below the diaphragm (T3b). However, patients with IVC involvement above the diaphragm (T3c) had significantly worse survival even after controlling for grade and performance status (PS) in a multivariate analysis. The 3-year DSS associated with RCC without thrombus, RV involvement (T3b), IVC involvement below the diaphragm (T3b), and IVC involvement above the diaphragm (T3c) were 60%, 36%, 35% and 12%, respectively. In addition, patients treated for metastatic RCC had a similar prognosis regardless of the level of venous involvement. These authors concluded that local tumor stage and grade were better predictors of prognosis than extent

of venous involvement, which supports the current TNM classification of venous involvement with RV and IVC invasion categorized as T3b and IVC involvement above the diaphragm categorized as T3c.

Several studies have also concluded that stage and grade of the tumor rather than the extent of the tumor thrombus determines prognosis [43,67,68]. Zisman et al. [69] compared 207 patients with tumor thrombus treated by nephrectomy and thrombectomy with 607 patients without tumor thrombus treated by nephrectomy. Involvement of RV and IVC was associated with significantly worse 5-year DSS (72% and 55%, respectively) compared to a survival rate of 88% for patients without tumor thrombus. For patients without nodal or distant metastases with IVC tumor thrombus, multivariate analysis revealed that capsular penetration, collecting system invasion, and extension into the hepatic vein were important prognostic variables, whereas the level of IVC thrombus (subdiaphragmatic, supradiaphragmatic, or atrial) was not [69]. In addition, a 5-year survival of 56% for patients with tumor thrombus extending into the right atrium was shown in one study [70]. These results suggest that while venous involvement is an indicator of poor prognosis, patients with venous involvement can be completely cured with aggressive surgical resection regardless of the extent of tumor thrombus extension. Recent studies have demonstrated 5-year survival to range from 47 to 69% for patients with venous involvement and tumor limited to the kidney [60,68-71], and with modern advances in surgical technique, surgical resection can be performed with acceptable morbidity [69]. In select patients with metastatic disease, resection of the tumor thrombus followed by immunotherapy has been recommended [72,73].

Invasion and infiltration of tumor into the wall of the vena cava has been reported in approximately 20% of patients, with the majority of tumors limited to the area of the RV ostium [71]. Surgical management of these tumors generally involves resection of the IVC and repair with a synthetic or autologous pericardium patch graft. This phenomenon may suggest a poor prognosis, but studies are limited. Although we have learned safe techniques for excising the thrombus, these patients are at high risk for later recurrence and death from metastases. Patients with venous involvement are ideal subjects for clinical trials testing new treatment strategies in the adjuvant setting, and they should be encouraged to participate in clinical trials.

### 1.5. Lymph node involvement

The overall risk of lymph node metastasis is approximately 20% and 5-year survival rates of patients with lymph node involvement range from 11 to 35% [74-76]. However, risk of lymph node involvement varies depending on primary tumor stage and size; vascular involvement; presence of metastases; and extent of lymphadenectomy performed [74,77]. Patients with clinically localized

disease have a relatively low incidence (2–9%) of nodal involvement [77], whereas in patients with metastatic disease or vascular involvement have an incidence as high as 45% [74]. Performing a lymphadenectomy could potentially cure someone with disease limited to the lymph nodes. However, no imaging modality currently exists that can accurately predict the presence of nodal metastasis. Several series have reported that in patients with lymph node positive disease, 30% had microscopic involvement only [76,78]. The false-negative rate for CT and MRI is low, with 4% of patients demonstrating positive nodes on lymphadenectomy [79]. However, specificity is poor as enlarged nodes seen on imaging is a poor predictor of nodal involvement as only 42% contained metastatic disease at the time of surgery [80].

Although it has been specified since the 6th edition of the TNM classification that histological examination of regional lymphadenectomy specimens should routinely include  $\geq 8$  lymph nodes, few studies have challenged the N1–N2 subclassification. Previous studies have focused on the number of lymph nodes that were required for accurate staging as well as the utility and extent of lymphadenectomy [81]. Terrone et al. [82] analyzed 618 patients who underwent lymphadenectomy to determine whether one or more than one positive lymph node was a relevant prognostic cut-off. The majority of patients (84.4%) underwent an extensive standardized lymphadenectomy that provided a median number of 13 lymph nodes for analysis. A significantly high percentage (49%) of distant metastases was associated with nodal invasion. There was no survival difference found between N1 (n = 29) and N2 (n = 59) tumors in patients with locally advanced or metastatic disease. Among patients with positive lymph nodes, the two relevant prognostic cut-offs were 4 involved nodes and a 60% lymph node density cut-off. In addition, lymph node density was retained as an independent prognostic variable. Furthermore, in the case of lymph node invasion, the median survival time was 14.4 months, even though an extensive lymphadenectomy was performed in most cases. Canfield et al. [83] analyzed the prognostic significance of nodal disease in the absence of distant metastatic disease. This study included a cohort of 40 patients with positive lymph nodes, but no systemic metastases, that underwent nephrectomy with extended retroperitoneal lymphadenectomy. Nodal status was N1 in 30% of patients and N2 in 70%. Extranodal extension was present in 70% of cases. Disease recurred in 28 patients (70%) and median time to recurrence was 4.9 months. Median survival in patients with N2 disease was significantly worse compared to patients with N1 disease (14.5 months vs. 35.7 months). On multivariate analysis,  $>1$  positive node was predictive of decreased DFS and overall survival. Dimashkieh et al. [84] examined the associations of pathological features of lymph node metastases with outcome in a cohort of patients treated with radical nephrectomy for unilateral, sporadic M0 RCC. There was no significant difference in survival between patients with N1 (n = 34) and N2 (n = 35) disease. However, patients

with extranodal extension were twice as likely to die of RCC compared to patients in whom metastases did not extend outside of the lymph node capsule. The 5-year DSS was 18% and 35% in patients with and without extranodal extension, respectively.

Pantuck et al. [74,75] evaluated the impact of lymphadenopathy in relation to response to immunotherapy and survival. In a review of 900 patients, positive lymph node status was associated with larger, higher grade, more locally advanced tumors more likely to demonstrate sarcomatoid features. Patients with lymphadenopathy were 3–4 times more likely to have distant metastatic disease. Patients with metastatic RCC with concomitant lymph node involvement demonstrated a significantly worse 5-year survival rate compared to patients with metastatic disease alone (15% vs. 23%). Similarly, patients with nodal disease manifested poorer response rates to immunotherapy. More importantly, patients with node-positive disease who underwent lymphadenectomy had better responses to immunotherapy and higher survival rates compared to patients whose involved lymph nodes were left in place [74,75]. This difference may be partly explained by the observation that positive lymph nodes rarely respond to immunotherapy [75]. Vasselli et al. [76] reported that patients with no preoperative evidence of lymph node involvement had a significantly longer median survival than those with lymph node involvement (14.7 months vs. 8.5 months). Patients with minimal or extensive lymphadenectomy had better survival than patients considered to have unresectable disease.

The practice of lymphadenectomy is lacking for an accepted standard as it is currently based largely on surgeon preference. The European Organization for Research and Treatment of Cancer (EORTC) conducted the only prospective, randomized controlled study (EORTC 30881) comprised of 772 patients with clinically localized disease randomized to nephrectomy with or without a standardized lymphadenectomy [79]. Although the data is still immature, there were no differences in progression or survival between patients treated with or without lymphadenectomy at 5-year median follow-up. However, the authors noted that the overall 5-year survival was high (82%) and longer follow-up is needed for more events to occur before a difference in survival may become apparent.

#### 1.6. Presence of distant metastases

Patients with metastatic RCC face a poor prognosis, with a median survival of 6–10 months and a 2-year survival of 10–20% [85]. Distant metastases can present anywhere throughout the body, but most frequently involve the lungs and bone [86]. Patients with bone metastases have been shown to have a significantly shorter median survival compared to those without [87,88]. In a cohort of 90

patients with metastatic disease, the mean survival for patients with bone metastasis was 13.8 months compared to 25.3 months for patients without bone metastasis [88]. More recent data demonstrates that the number of metastatic sites of RCC rather than actual location dictates the overall prognosis. Han et al. [86] evaluated 434 patients with node-negative metastatic RCC and divided patients by organ involvement. Patients with more than one metastatic site had a lower response rate to immunotherapy following nephrectomy of the primary tumor and a significantly shorter survival than patients with a single metastatic site. For patients with lung-only, bone-only, and multi-organ involvement, the mean survival was 27, 27, and 11 months, respectively. Multivariate analysis confirmed that metastatic disease to more than one organ site was associated with poor prognosis. Toyoda et al. [89] retrospectively analyzed the survival of patients with RCC and bone metastases. Median survival time from the diagnosis of bone metastasis was 12 months, and overall survival at 2 years was 37%. Clinical features that correlated with longer survival in the multivariate analysis were a long interval ( $\geq 24$  months) between the diagnosis of kidney cancer and that of osseous metastasis and the absence of extraosseous metastases.

## 2. Role of multicenter databases for improving TNM predictive accuracy

TNM classification needs continuous assessment and improvements. Since large multicenter databases reflect the worldwide reality, they are very useful both for questioning the pertinence of the TNM classification and for improving its predictive accuracy. Recently, the main TNM modifications that were proposed were based on integration of information on tumor size for organ-confined tumors [31]. Therefore, a 4 cm tumor size break point was decided separating T1 tumors in T1a and T1b tumors. Although it was subject to controversy, this tumor size cut-off was chosen based on previous survival studies along with the perception that this cut-off was also appropriate for choosing between conservative and radical nephrectomy options. In a series including 2217 patients with localized RCC from 7 European Centers, it was confirmed that the T1a–b subclassification was able to stratify patients accurately for prognosis [90]. Moreover, in a series including 1454 patients with T1N0M0 tumors, it was shown that in selected patients, partial nephrectomy compared to radical nephrectomy provided similar cancer control in tumors measuring more than 4 cm [33]. In a more recent study including 2245 patients with clear cell histology, it was confirmed that tumor size when used as a continuous variable added some prognostic information to TNM classification [91]. Interestingly, the resulting TNM predictive accuracy improvement was more sensible for the 1997 TNM classification than for the 2002 classification, probably due to the fact that the more recent TNM version had already included refinement in tumor size as prognostic information.

Another way to try to improve the TNM system is to include new variables within the classification. The ideal prognostic variable has to be universally and easily available. In the metastatic setting, it is well accepted that PS is a strong predictor for survival. Similarly, many recent studies have shown that cancer related symptoms were independent prognostic parameters in localized RCC [92-94]. Recently, a symptom-based classification was established and externally validated in a large multicenter series [95,96]. Additionally, it was demonstrated in two different large subsets of patients that integrating both tumor size and tumor-related symptom information within the TNM classification resulted in improved prognostic stratification [97, 98].

## 2. Validated non-anatomical prognostic factors

RCC was historically regarded as a single entity that expressed many possible histological appearances. RCC is now more accurately recognized as a family of cancers resulting from distinct genetic abnormalities with unique morphological features, but a common derivation from the renal tubular epithelium. Aside from the known anatomical prognostic variables, numerous histological as well as clinical criteria have been shown to impact prognosis in patients with RCC.

### 2.1. Nuclear grade

Nearly all histopathological tumor grading systems have shown independent prognostic value in studies that included grade as a variable. Fuhrman et al. [99] developed a four-tier grading system based on nuclear and nucleolar size, shape, and content (Figure 1).

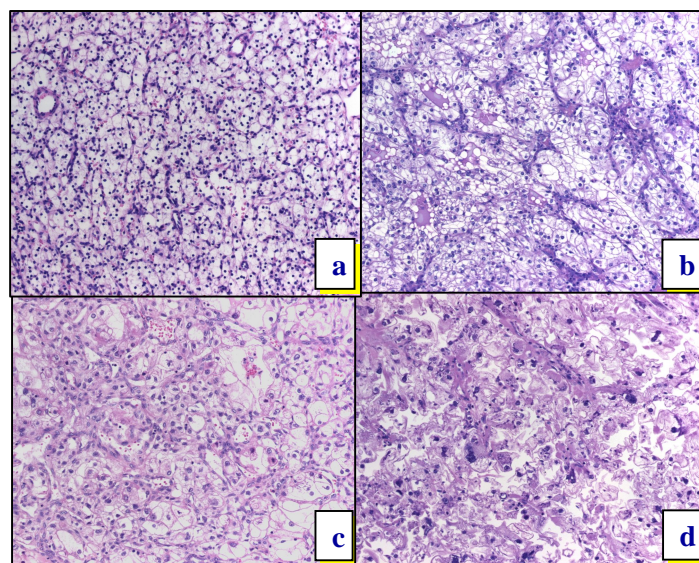


Figure 1: Fuhrman Nuclear grade: grade 1 (a); grade 2 (b); grade 3 (c); grade 4 (d).

Nuclear grade has been shown to correlate with tumor stage, tumor size, metastases, lymph node involvement, vascular involvement, and perirenal fat involvement [100]. Unfortunately, controversy exists concerning the interobserver reproducibility of grading and relevant breakpoints between the different grades and survival. Tsui et al. [38] demonstrated a strong correlation between tumor grade and survival with 5-year Disease-specific Survival (DSS) rates of 89%, 65%, and 46.1% for grades 1, 2, and 3–4, respectively. Furthermore, 5-year DSS rates in patients with T1 tumors were 91%, 83%, 60%, and 0% for grades 1, 2, 3, and 4, respectively, demonstrating that histologic grade was an independent prognostic indicator even among patients with the same anatomic stage of RCC.

## 2.2. Histologic subtype

The current classification system is based on conclusions developed by the UICC and the American Joint Committee on Cancer (AJCC) [101]. The cellular origin of RCC accounts for the histological variations of RCC. Of the four main subtypes of RCC, clear cell is the most common, accounting for 70–80% of cases. Papillary RCC accounts for 10–20% and is divided into two types based on morphological appearance. Type 2 papillary RCC has been found to behave more aggressively than type 1, and a recent analysis further demonstrated that type 2 papillary RCC was a significant and independent predictor of poor survival [102]. Chromophobe RCC accounts for approximately 5% of cases. Several large studies have demonstrated that patients with chromophobe RCC have an excellent prognosis and overall survival of patients with chromophobe RCC seems to be better than patients with other types of RCC [103,104]. However, chromophobe and papillary subtypes of RCC are associated with extremely poor responses to IL-2 therapy, with median survival times reported to be 11 months and 5.5 months, respectively [105]. The question of whether different histological variants of RCC portend different survival outcomes remains controversial. A retrospective review of 2385 RCC patients treated by radical nephrectomy at the Mayo Clinic, demonstrated worse DSS for clear cell RCC compared to papillary or chromophobe RCC, with 5-year DSS rates of 69%, 87%, and 87%, respectively [106]. This survival difference persisted after stratifying for TNM stage and grade. However, a recent international, multicenter study of 4063 RCC patients treated by surgical resection reported that the prognosis of patients with chromophobe RCC was better than those with clear cell or papillary RCC, but they all had equivalent survival when adjusted for stage and grade, and in multivariate analysis, histologic subtype was not retained as an independent prognostic factor affecting survival [107]. Collecting duct carcinoma is an aggressive and rare variant of RCC accounting for less than 1% of tumors [108,109]. Renal medullary carcinoma is a



subgroup of collecting duct carcinoma almost exclusively diagnosed in young black men with sickle cell trait or sickle cell disease and has a poor prognosis [110].

Regardless of the presumed prognostic value, tumor subtype is considered as a parameter of significant importance in distinguishing categories with different morphological, molecular and genetic characteristics and it is a matter of indisputable guide for the correct evaluation of other histopathological grading in which nuclear and the coagulation necrosis in the context of each category.

### 2.3. Sarcomatoid features

Sarcomatoid RCC was first described in 1968 as a separate variant that contained highly pleomorphic spindle cells [111]. The sarcomatoid variant is no longer considered a distinct histological subtype of RCC [112], rather it represents a relatively rare, high grade form of RCC typified by a spindle cell growth pattern evident in less than 5% of RCC cases and is associated with a poor outcome [113]. Sarcomatoid features can be seen in any of the histologic variants of RCC. de Peralta-Venturina et al. [114] recently reviewed 101 cases of RCC with sarcomatoid features and reported 5- and 10-year survival rates of 22% and 13%, respectively.

### 2.4. Tumor necrosis

Histologic necrosis is defined as any degree of microscopic tumor necrosis exclusive of degenerative changes such as hyalinization, hemorrhage, or fibrosis. The presence of tumor necrosis has been recognized to be associated with markers of advanced disease [39, 103, 115]. Amin et al. [103] analyzed the prognostic value of histologic necrosis in 405 RCC specimens and multivariate analysis revealed that TNM stage, grade, and histologic necrosis were associated with survival. Patients with necrosis in the pathology specimen had a 3-fold higher likelihood of death owing to RCC when compared with patients without histologic necrosis. In 1801 patients at the Mayo Clinic with unilateral clear cell RCC, presence of histologic necrosis in tumor specimens was shown to be an independent predictor of survival associated with twice the risk of death from RCC compared to patients without necrosis [39]. Moch et al. [104] found that the presence of tumor necrosis was an independent predictor of poor outcome in clear cell, but not papillary RCC. Sengupta et al. [116] evaluated 3009 patients undergoing radical or partial nephrectomy for RCC. Necrosis was observed in 28%, 47%, and 20% of clear cell, papillary, and chromophobe subtypes, respectively. Histologic tumor necrosis was found to be a strong independent predictor of outcome for clear cell, but not for chromophobe or papillary RCC [39, 116]. Lam et al. [115] reported in 310 patients with localized

or metastatic RCC that both the presence and extent of histologic necrosis in tumor specimens were independent predictors of survival only in patients with localized disease. Furthermore, tumor necrosis was associated with markers of poor outcome including lymph node involvement, presence of distant metastases, greater tumor size, and higher grade.

## 2.5. Collecting system invasion

Uzzo et al. [117] reviewed 426 nephrectomy specimens and determined that the overall incidence of collecting system involvement was 14%. Tumors with collecting system involvement were associated with clear cell histology, higher grade, higher TNM stage, and larger size. Collecting system invasion in high stage tumors (T3 or greater) did not affect prognosis, whereas in low stage tumors (T1 and T2) collecting system involvement was associated with poor prognosis with a median survival of only 46 months. Palapattu et al. [118] reviewed the records of 895 patients who underwent nephrectomy and also observed a 14% incidence of collecting system involvement. Patients with collecting system invasion were associated with symptoms, higher T-stage, and presence of metastatic disease. There was no difference in the incidence of collecting system invasion seen between histological cell types. Patients with collecting system invasion had a significantly lower 3-year DSS compared to patients who did not have involvement (62% vs. 39%). This difference was particularly evident in patients with stage T1 tumors (81% vs. 67%). Furthermore, multivariate analysis demonstrated collecting system invasion to be an independent predictor of survival associated with a 1.4-fold greater risk for death when compared with patients without collecting system invasion. Terrone et al. [119] analyzed 671 tumors and found that patients with tumors invading the collecting system were usually symptomatic and associated with higher grade and tumor stage. The 5-year DSS appeared to be only significantly different for T2 tumors (33% vs. 77%). However, collecting system invasion was not an independent predictor of outcome.

## 2.6. Microvascular invasion

Microvascular invasion (MVI) can be seen on histologic analysis when tumor cells invade into the walls of small vessels. As tumor cells are directly accessible to the vasculature, hematologic shedding is a plausible pathway for distant metastasis. The incidence of MVI in RCC has been reported to be 12–28% [120-122]. It was previously believed that MVI did not play a prognostic role in RCC. However, recent data may prove otherwise. Van Poppel et al. [120] assessed 180 patients undergoing nephrectomy. At 4-year follow-up, 39.2% of patients with MVI demonstrated disease progression compared to 6.2% of patients without MVI. In this small cohort, multivariate

analysis determined that MVI was the most important independent prognostic factor in DFS. Goncalves et al. [121] evaluated a cohort of 95 patients undergoing nephrectomy for T1 and T2 tumors. MVI was associated with poor prognostic features such as large tumor size, perirenal fat involvement, high grade, lymph node involvement, and sarcomatoid features. MVI was also found to be an independent predictor of disease recurrence and DSS [121]. Lam et al. [122] recently evaluated the prognostic significance of MVI in patients with clear cell RCC. Patients with MVI were more likely to present with symptoms related to their disease, higher T-stage, lymph node involvement, distant metastases, higher grade tumors, larger tumor size, and positive surgical margins. The 5-year DSS was significantly poorer in patients with MVI (27% vs. 61%).

## 2.7. Performance status

The Karnofsky or Eastern Cooperative Oncology Group (ECOG)-PS scales are a convenient common denominator of the overall impact of multiple objective and subjective symptoms and signs on patients and are often used as eligibility criteria for immunotherapy regimens and entry into clinical trials. The ECOG-PS scale is a four point scoring system is rated 0–4 stratifying patients based on ambulatory status used to denote impact of disease on the overall health of the patient. The Karnofsky scale is a similar scale, but differs from ECOG-PS in that patients with higher Karnofsky scores have improved ambulatory status. Several studies have demonstrated the ECOG-PS to be an independent prognostic factor of survival in patients with metastatic RCC at presentation with higher scores correlating with poorer survival [87]. As such, clinical trials have incorporated low ECOG-PS score into the inclusion characteristics for IL-2-based therapies [123]. Tsui et al. [38] evaluated the role of ECOG-PS in all stages of RCC and found that poor PS was an independent predictor of poor outcome. The 5-year DSS for patients with ECOG-PS score 0 was 81% compared to 51% for those with ECOG-PS score  $\geq 1$ . PS has also been shown to be an important predictor of bone metastasis in patients presenting with presumed RCC lesions [124]. Only 1.4% of patients with an ECOG-PS score of 0 harbored bone metastasis, of whom 71% complained of musculoskeletal pain, 100% manifested extraosseous metastases and 25% had increased alkaline phosphatase at presentation. Based on these findings, the authors recommended performing a bone scan in patients with an ECOG-PS score  $\geq 1$  regardless of T-stage.

## 2.8. Paraneoplastic symptoms

Localized RCC rarely causes symptoms and metastatic RCC may result in signs and symptoms specific to the site of metastatic disease. However, it has been reported that approximately 20% of all patients diagnosed with RCC present with paraneoplastic symptoms [125]. Another 10–40% of

patients will develop paraneoplastic symptoms during the course of their disease. Paraneoplastic findings are distinct from the sequelae of local or distant invasion by RCC. They represent a constellation of signs and symptoms that result from the humoral release of various tumor-associated proteins. The proteins responsible for the paraneoplastic effects may be elaborated directly by the tumor cells or by the immune system in response to the tumor. As a rule, paraneoplastic effects resolve following complete surgical resection of the tumor.

Paraneoplastic syndromes include a long list of varied signs and symptoms. In a modern series of RCC patients, the most common findings at the time of presentation were anemia, hepatic dysfunction, unintended weight loss, malaise, hypoalbuminemia and hypercalcemia [125]. These findings likely represent paraneoplastic effects; however, confirmation of a paraneoplastic effect requires documentation of resolution of the sign or symptom following complete resection. The majority of the findings occurred more frequently in patients with localized rather than metastatic RCC, suggesting that these findings reflect tumor biology rather than simply the local or distal extent of the tumor. In a univariate analysis, all but the rarest findings portended a significantly worse prognosis. The highest hazard scores were associated with anorexia, unintended weight loss, malaise, thrombocytosis and night sweats.

Kim et al. [125] examined a cohort of 1046 patients with localized and metastatic RCC undergoing nephrectomy and of the paraneoplastic effects examined, hypoalbuminemia, weight loss, anorexia and malaise were identified as predictors of poor prognosis that were independent of stage, grade and PS. All four independent predictors of survival were also signs and symptoms associated with tumor cachexia; therefore, the term cachexia-related finding was coined in the study. Although there was a trend towards worse survival with increasing numbers of cachexia-related findings, the difference in survival between patients with one versus all four cachexia-related finding was not statistically different. In patients with localized RCC, the 2-year survival rate was significantly higher in patients without cachexia compared to patients with at least one cachexia-related finding (95% vs. 79%). In patients with metastatic RCC, the median survival was significantly worse in patients with cachexia compared to patients without cachexia (12 months vs. 31 months). The authors recommended incorporating cachexia-related findings into the measurement of ECOG-PS to give a more accurate representation of PS.

Cachexia is a universal finding in patients with widely metastatic and advanced malignancies related to high tumor burden. Therefore, in order to better assess whether cachexia reflects tumor biology in addition to tumor burden, patients with localized T1 RCC were evaluated in a separate study. For patients with localized disease, cachexia is presumably related to the paraneoplastic effects of secreted tumor molecules. In this subgroup, at least one of the cachexia-related findings

was found in approximately 15% of the patients [126]. Cachexia was an independent predictor for both tumor recurrence following nephrectomy and poor survival. The effect of cachexia on survival rates was most pronounced for patients with high grade RCC where the presence and absence of cachexia were associated with 5-year survival rates of 55% and 75%, respectively.

Several studies have compared the prognostic significance of symptomatic presentation of RCC. In these studies, all patients with a variety of symptoms were grouped together. Two previous reports concluded that there was no difference in prognosis for patients with RCC presenting with or without symptoms [127,128]. However, others have reported that symptomatic presentation portends a worse prognosis [129-131]. These conflicting results suggest that specific signs and symptoms (i.e. cachexia-related findings), and not simply the presence or absence of symptoms, have a prognostic significance.

## 2.9. Thrombocytosis

The presence of thrombocytosis (platelet count  $>400,000 \text{ mm}^{-3}$ ) has been shown to be an independent predictor of poor outcome in patients with metastatic RCC treated with nephrectomy and various adjuvant therapies. Patients with thrombocytosis have a lower mean survival compared to those without thrombocytosis (18 months vs. 34 months) [132]. The presence of thrombocytosis in localized RCC has also been shown to be important in outcome following nephrectomy. Gogus et al. [133] examined a cohort of 151 patients that underwent radical nephrectomy for localized RCC. Preoperative thrombocytosis was associated with higher T-stage and nodal involvement. The presence of thrombocytosis had a worse DSS compared to patients without thrombocytosis (45.2 months vs. 76.6 months). Bensalah et al. [134] recently evaluated the prognostic value of thrombocytosis in localized and metastatic RCC from a review of 804 patients. Platelet count was strongly correlated with T-stage, grade, tumor size, RV involvement, perinephric fat invasion, nodal involvement, and presence of distant metastasis. TNM stage, grade, tumor size, ECOG-PS, and platelet count were significant predictors for survival following multivariate analysis. The 5-year survival rate for platelet counts  $<450,000 \text{ mm}^{-3}$  was 70% compared to 38% for platelet count  $\geq 450,000 \text{ mm}^{-3}$ .

## 2.10. Inflammatory response

It is recognized that in addition to tumor stage, disease progression is dependent on a complex interaction of the tumor and host inflammatory response [135-137]. Serum C-reactive protein (CRP) has been shown to be associated with prognosis in patients with advance RCC [138,139].

Fujikawa et al. [138] demonstrated that patients with an elevated pretreatment serum CRP ( $\geq 1.0$  ng/mL) who underwent surgery had a better prognosis compared to those who did not undergo surgery. In addition, survival was prolonged in patients in whom postoperative nadir CRP decreased to within normal limits when surgery was combined with postoperative immunotherapy. High CRP is caused by excessive IL-6 production, a multifunctional cytokine with growth factor function in RCC [140]. IL-6 has been shown to correlate with stage, grade, and proliferative index [141]. Negrier et al. [142] also demonstrated that IL-6 significantly correlated with progression-free survival and overall survival in patients with metastatic RCC, as well as an independent prognostic factor for overall survival. Blay et al. [143] showed that pretreatment concentrations of IL-6 and CRP were higher in patients with metastatic RCC who experienced progressive disease after IL-2 treatment. Lamb et al. [144] also recently demonstrated that CRP and the UCLA integrated staging system (UISS) were significant independent predictors of survival in patients who have undergone potentially curative resection for clear cell RCC. CRP has also been shown to hold prognostic value in patients with localized disease [145,146]. Komai et al. [145] reported that the 5- and 10-year DSS rates (75% and 30%, respectively) in patients with high CRP levels were significantly worse than those in patients with normal CRP levels (both 93%). Furthermore, pathological stage and an elevated CRP were the most important prognostic factors for DSS in patients with localized RCC. Erythrocyte sedimentation rate (ESR) is largely regarded as a non-specific marker of inflammation. The prognostic utility of ESR in patients with RCC has been previously evaluated [147-151]. Lehmann et al. [150] reported the best predictor of progression-free survival and DSS in patients with non-metastatic RCC after tumor size and symptomatic presentation was ESR, with ESR values  $>70$  mm at 2 h indicating a significantly poorer prognosis. Sengupta et al. [151] recently reported that an elevated ESR in patients with RCC suggested the presence of aggressive disease and poorer outcomes after surgical treatment. In addition, the association between an elevated ESR and death from clear cell RCC persisted even after multivariate analysis.

### 3. Comprehensive integrated staging systems and predictive nomograms

The anatomical, histological, and clinical factors that influence disease recurrence and survival in RCC make counseling patients particularly challenging.

Many Authors in last few years have proposed the use of mathematical models based on the integration of the main prognostic factors with the aim to improve the prognostic accuracy of individual clinical or pathological variables previously considered. The main prognostic integrated systems available today enable you to estimate the progression-free survival or cause-specific in patients with localized or metastatic disease [9]. Their main use would affect the planning of post-

operative follow-up, counseling of patients and the definition of criteria for the inclusion and evaluation of the results of clinical trials.

Many centers have aimed to integrate these independent prognostic indicators into comprehensive outcome models for both non-metastatic and metastatic RCC to assist clinicians in facilitating patient counseling and identifying those patients who might benefit from treatment [39,41,131,152-162]. The factors predicting outcome for patients with metastatic disease included PS, presence of metastases, and metastatic-free interval. More recently, several groups have created similar models designed to include patients with localized and metastatic disease.

### 3.1. Eastern Cooperative Oncology Group

In 1988, Elson et al. [152] presented an analysis of 610 patients with recurrent or metastatic RCC who had been treated with chemotherapy in clinical trials sponsored by ECOG. A scoring system was developed to stratify patients into 5 categories based on ECOG-PS (0–1 vs. 2–3), time from initial diagnosis (>1 year vs. 1 year), number of metastatic sites, prior cytotoxic chemotherapy, and recent weight loss. Using this system, median survival times ranging from 2.1 to 12.8 months were observed across the five separate categories. As this cohort was performed prior to the initiation of immunotherapy, its validity for today's patient population is questionable.

### 3.2. Memorial Sloan-Kettering Cancer Center (Motzer criteria)

Motzer et al. [153] developed a model based on 670 patients with advanced RCC treated in 24 separate clinical trials at Memorial Sloan-Kettering Cancer Center (MSKCC), including 394 patients treated with IFN- $\alpha$  or IL-2. This model was created by defining the relationship of pretreatment clinical features and survival, which included such risk factors as low Karnofsky PS score, high serum lactate dehydrogenase levels, low hemoglobin levels, hypercalcemia, and prior nephrectomy. Median survival was 10 months and significantly shorter survival occurred in patients with poor PS (Karnofsky scale <80%), high lactate dehydrogenase (>1.5 times upper limit of normal), low hemoglobin, high-corrected calcium (>10 mg/dL), and absence of prior nephrectomy. Patients were stratified into favorable-, intermediate-, and poor-risk groups according to the number of risk factors present. Patients at poor risk with 3 or more risk factors had a median survival of 4 months, whereas median survival improved to 20 months in those with no risk factors.

To analyze prognostic factors that would benefit modern day clinical trials, Motzer et al. [163] recently reviewed 137 patients with metastatic RCC enrolled in clinical trials at MSKCC from 1990 and onwards. Median overall survival of this group was 12.7 months. Independent predictors of worse survival were poor PS (Karnofsky scale <80%), low hemoglobin (less than or equal to 13

g/dL in males and 11.5 g/dL in females), and elevated corrected serum calcium ( $\geq 10$  mg/dL). The number of poor prognostic variables stratified patients into favorable- (no risk factors), intermediate- (one risk factor), and poor-risk groups (two or three risk factors). Favorable-, intermediate-, and poor-risk groups demonstrated overall 1- and 3-year survival rates of 76% and 25%, 49% and 11%, and 11% and 0%, respectively.

### 3.3. Kattan postoperative prognostic nomogram

The Kattan postoperative prognostic nomogram [131] was created to predict the probability of tumor recurrence within 5 years in patients undergoing radical nephrectomy for RCC. The nomogram assigns numerical scores to various prognostic indicators, such as the presence of symptoms, histology, tumor size, and the standard TNM staging criteria. In a study of 601 patients with RCC who were treated with nephrectomy, the nomogram appeared accurate and discriminating, with an area under the receiver operating curve of 0.74 [131]. This nomogram was later modified to exclude histologic subtype and analysis was limited to clear cell RCC [164]. The 5-year probability of freedom from recurrence for the patient cohort was 80.9%. This nomogram has a concordance index of 0.82 and external validation revealed it to be accurate and discriminating.

### 3.4. Mayo Clinic stage, size, grade, and necrosis (SSIGN) scoring algorithm

The Mayo Clinic created an extensive outcome prediction model only for patients with clear cell RCC undergoing radical nephrectomy [39]. According to an analysis of data from 1801 patients, TNM stage, tumor size  $< 5$  cm, nuclear grade, and the presence of histological tumor necrosis were all found to be independent predictors of survival. These factors were combined into the stage, size, grade, and necrosis (SSIGN) scoring algorithm. Decreased survival was shown to correlate with increased SSIGN score, with scores of 0–1 and  $> 10$  correlating with 5-year DSS rates of 99% and 7%, respectively.

### 3.5. University of California-Los Angeles integrated staging system (UISS)

This UISS is an extensive prognostic system that has been created for both localized and metastatic RCC [154]. The initial UISS contained 5 groups based on TNM stage, Fuhrman grade, and ECOG-PS. Projected 2- and 5-year survival for patients in UISS groups I–V were: 96% and 94%, 89% and 67%, 66% and 39%, 42% and 23%, and 9% and 0%, respectively. The UISS was internally validated using a bootstrapping technique and then using an expanded database of patients treated at



UCLA between 1989 and 2000 [165], with external data from patients treated at MD Anderson Cancer Center and in Nijmegen, Netherlands [166], and most recently with 4202 patients from 8 international centers [167]. The UISS has been subsequently modified into a simplified system, based on separate stratification of metastatic and non-metastatic patients into low-, intermediate-, and high-risk groups [168]. This provides a clinically useful system for predicting postoperative outcome, and provides a unique tool for risk assignment and outcome analysis to help determine follow-up regimens and eligibility for clinical trials.

### 3.6. Cleveland Clinic

A study of 353 patients with previously untreated advanced RCC at the Cleveland Clinic was conducted to assess and validate the model proposed from MSKCC [156]. Four (time from diagnosis to entry onto study, serum lactate dehydrogenase, corrected serum calcium, and hemoglobin) of five prognostic factors identified by the MSKCC group were independent predictors of survival. In addition, prior radiotherapy and presence of hepatic, lung, and retroperitoneal nodal metastases were found to be independent prognostic factors. Using the number of metastatic sites as surrogate for individual sites (none or one vs. two or three sites), the MSKCC definitions of risk groups were expanded to accommodate these two additional prognostic factors. Using this expanded criteria, favorable-risk was defined as zero or one poor prognostic factor, intermediate-risk as two poor prognostic factors, and poor-risk as more than two poor prognostic factors.

### 3.7. International Kidney Cancer Working Group

The International Kidney Cancer Working Group is currently establishing a comprehensive database from centers that treat patients with metastatic RCC. This will be used to develop a set of prognostic factors in patients with metastatic RCC and ultimately to derive a single validated model. Initially as part of this project, preliminary studies were performed to determine the availability of a database that could be used for the planned analysis of prognostic factors, which involved the examination of 782 patients treated by the Groupe Francais d'Immunotherapie [158] and patients treated at the Cleveland Clinic [156]. These two groups were similar in their distribution of various clinical factors and survival, which demonstrated the similarities between these two patient populations [159]. These findings suggest that use of an international database would be a reasonable approach to identify prognostic factors and validate a model for patients with this disease. Additionally, this database could serve as a resource to study the natural history of this illness and aid in design and analysis of clinical trials for patients with metastatic RCC.

### 3.8. Providing external validation of prognostic systems

Although the TNM system is a universal tool for prognostication it has some limitations particularly due to its simplicity which do not reflect the biologic complexity of renal tumors. Recently, some new prognostic systems combining independent prognostic variables have been designed [169]. So far, very limited data exist about the practical use of such systems. The only prognostic system that has been externally validated through large external multicenter series is the UISS system [167,166]. In 4202 patients from 8 academic centers from Europe and United States, it was established that the UISS system was able to stratify patients both in localized and in metastatic setting. Particularly in localized disease, the UISS worked perfectly well in all centers having different types of recruitment. Recently, the SSIGN system has also been externally validated in a single center series [170]. Moreover, large multicenter databases are not only useful for validating pre-established prognostic systems, it also allows the creation of new prognostic tools [171] and the comparison of prognostic systems together [172]. A study from 6 European centers with 2404 patients attempted to externally validate four prognostic models (Kattan nomogram [131], Yacyioglu model [173], Cindolo model [171], and the UISS [154]) and compare the accuracy in discriminating outcome for non-metastatic RCC [172]. Each of the models validated their ability to distinguish groups with different outcome and the postoperative models, Kattan nomogram and UISS, appeared to better predict outcome compared to the preoperative models, Yacyioglu and Cindolo models. Both the Kattan nomogram and UISS worked well, although the Kattan nomogram was slightly more accurate improving discrimination in the UISS intermediate-risk patients [172]. However, the authors concluded that both models were most likely the same on practical grounds. Since many patients have only a partial regression of metastases or prolonged stabilization following systemic therapy, surgery must often be considered. Clinical situations in which surgery is potentially appropriate include: excision of locally recurrent disease, excision of solitary or multiple metastases, resection of a residual mass after systemic therapy, and palliation. Several series have demonstrated a potential benefit for complete surgical resection of all tumor burden, including removal of both the primary renal mass as well as metastatic deposits in carefully selected patients with minimal volume metastatic disease. Surgical removal of solitary metastasis is widely accepted as potentially effective with 5-year overall survival rates of 29–35% [174-179]. Best results are obtained if the metastases are pulmonary, metachronous with a long disease-free interval and completely resected [179,180]. Kavolius et al. [174] have reported on the largest single series of patients undergoing resection of a solitary metastasis. Patients with completely resected solitary metastasis fared better than those who underwent complete resection of multiple metastases, with a 5-year overall survival of 52% compared with 29%. Patients who present with synchronous

metastases have an average survival time of approximately 4 months with only 10% surviving 1 year, whereas patients with metachronous metastases have a median survival time of 11 months regardless of the site of metastasis [181,182].

#### 4. Molecular biomarkers for diagnosis, prognosis, and therapy

Molecular biomarkers may prove more effective for predicting survival than traditional clinical parameters such as tumor stage and grade [123]. Recently, methods based on gene arrays, which screen for differential expression of thousands of genes, have identified large numbers of potential prognostic biomarkers [184-186]. These biomarkers require validation on larger patient populations, and validated biomarkers can be integrated into a multimarker prognostic system. A useful tool for validating a limited number of biomarkers on a large patient population is the tissue microarray (TMA). To construct an array, cores (6 mm, diameter) of tissue from archival blocks are precisely arrayed onto a recipient paraffin block. Multiple cores representing the heterogeneity of the tumor can be arrayed from each patient tumor. Each receipt block can contain up to 500 cores, which can be cut onto a glass slide for standard immunohistochemical staining. TMA allows large numbers of samples to be uniformly stained using a small amount of antibodies, and facilitates review of the staining by the pathologist.

The evaluation of protein expression in a high-throughput TMA is a natural extension to the efforts for molecular staging. Sections of the TMA provide targets for parallel in situ detection of DNA, RNA and protein in the same set of specimens, which can be correlated to clinical data with respect to disease progression, treatment response, and survival. By better understanding the genetic alterations at the molecular level, clinicians will be able to tailor treatment. These tumor-specific treatment plans will be more effective at targeting the genotypic alterations unique to each cancer with less systemic side effects. Currently, there are a variety of molecular targets that have been identified to be important in RCC prognosis and specific therapies have been created to combat these alterations [6].

##### 4.1 Von Hippel-Lindau gene

Identification of the Von Hippel-Lindau (VHL) gene and understanding the function of this pathway in RCC tumorigenesis has played a major role in the development of RCC therapeutics. VHL disease is a hereditary cancer syndrome that has proven to be highly informative with respect to the pathogenesis of clear cell RCC. In affected families, cancer risk is transmitted in an autosomal dominant manner on chromosome 3p, and the syndrome is manifested by retinal angiomas, central nervous system hemangiomas and clear cell RCC [187,188].

Individuals with VHL disease carry in their germline one wild-type VHL allele and one inactivated VHL allele. Pathologic changes ensue in VHL disease when the remaining wild-type allele is somatically inactivated in a susceptible cell type. Therefore, VHL is a classic two-hit tumor suppressor gene [188]. It has been shown that inactivation of the VHL gene is an early step in the development of clear cell RCC associated with VHL disease [188]. The VHL tumor suppressor gene is mutated in all hereditary RCC and approximately 50% of sporadic RCC, and thus the majority of clear cell

RCCs appear to be linked to biallelic VHL inactivation [189]. The majority of clear cell RCC demonstrates either a mutation of the VHL gene or downregulation of its protein product. Translation of VHL mRNA gives rise to protein that is referred to generically as VHL protein (pVHL), which has an important role in the cellular response to hypoxia [190].

Among the many functions attributed to pVHL, the one most clearly linked to the development of RCC is inhibition of hypoxia-inducible factor (HIF) [191].

HIF is a heterodimeric transcription factor consisting of an unstable  $\alpha$ -subunit (HIF1 $\alpha$ ) and a stable  $\beta$ -subunit [192].

Under normal oxygen conditions, the pVHL complex polyubiquitinates HIF1 $\alpha$ , tagging it for destruction by the proteasome [193]. Under low oxygen conditions or in cells lacking pVHL, HIF1 $\alpha$  accumulates, binds to HIF1 $\beta$  and transcriptionally activates hypoxia-inducible genes [192].

The consequence of mutated pVHL is similar to that of cellular hypoxia causing HIF dimerization and stabilization. During hypoxia, there is accumulation of hydroxyl-free HIF that no longer binds to VHL. HIF1 $\alpha$  is stabilized by dimerization with the constitutively expressed HIF1 $\beta$  subunit and translocates to the nucleus. The HIF1 $\alpha$  and HIF1 $\beta$  complex binds to HIF inducible gene promoter regions, including genes implicated in angiogenesis, pH regulation, glycolysis, glucose transport, cell cycle, chemotaxis, signaling, and apoptosis [194].

## 4.2 Angiogenesis

### 4.2.1 VEGF-R pathway

Vascular endothelial growth factor (VEGF, also known as vascular permeability factor and VEGF-A) is a dimeric glycoprotein and a member of the PDGF superfamily of growth factors that includes VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor. VEGF is a tumor-secreted cytokine with critical importance in both normal and tumor-associated angiogenesis, as well as lymphangiogenesis, and is a downstream product of the HIF pathway [195]. In addition to increasing microvascular permeability to plasma proteins [196], VEGF has been further

characterized with regard to multiple effects relevant to the generation and preservation of tumor vasculature that induction of endothelial cell division and migration [197,198], promotion of endothelial cell survival through protection from apoptosis [199], and reversal of endothelial cell senescence [200].

Biallelic loss of VHL leads to upregulated transcription of growth factors such as VEGF, PDGF and TGF- $\alpha$ . These factors bind to their respective receptors (VEGF-R, PDGF-R and EGF-R), which are each tyrosine kinase receptors. Consequent binding of ligands to these receptors leads to downstream signaling that results in increased cell proliferation, upregulated angiogenesis and decreased apoptosis. RCC associated mutations of pVHL are invariably inactivating the process of HIF destruction, suggesting that HIF plays a critical role in RCC carcinogenesis. Numerous HIF responsive genes have been described, with a number of these genes encoding proteins that are growth factor receptors or their ligands, some of which were listed above [201].

A number of HIF responsive gene products are implicated in tumorigenesis. Uncontrolled production of these growth factors provides a stimulus for the tumor and endothelial cell proliferation. Angiogenic stimuli produced secondary to metabolic demands of host tissues initiate the angiogenic response in healthy individuals. Upon binding to membrane receptors in vascular endothelial cells, a five-step process is triggered. Initially, the vascular endothelial basement membrane of the parent vessel breaks down, allowing a route for the development of a new capillary sprout. This is followed by migration of endothelial cells and chemoattraction [202].

This leading front of migrating cells is driven by enhanced proliferation of endothelial cells, followed by formation of capillary tubes via organization of the endothelial cells, and a recruitment of pericytes and vascular smooth muscle cells for capillary stabilization [203]. During tumorigenesis, the angiogenic switch is activated directly via induction of angiogenic growth factors or indirectly by recruiting host immune cells that release mediators of angiogenesis [204].

Induction of the HIF pathway results in production of VEGF. VEGF a key regulator of angiogenesis and functions are mediated through two tyrosine kinase receptors, VEGF-R1 and VEGF-R2, in vascular endothelial cells [205,206]. VEGF initially interacts with VEGF-R2 to promote endothelial cell proliferation, migration and vascular permeability, and subsequently activates VEGFR1 to assist in the organization of new capillaries. Several therapeutics targeting angiogenic pathways are currently being evaluated in clinical trials for their efficacy and long-term clinical benefits, while others are being mechanistically exploited toward the development of novel therapeutic modalities for treating RCC.

Understanding the expression of the VEGF-VEGF receptor (VEGFR) family will individualize the selection of target-specific therapies based on the tumor biology and optimize the benefit of agents

that target these pathways in RCC. Ljungberg et al. [207] demonstrated different patterns of expression of VEGF and VEGFR mRNA levels between the different RCC histological subtypes and were associated with tumor stage and survival. In patients with clear cell RCC, VEGF mRNA levels below the median had a significantly shorter survival time compared to those with higher levels. By contrast, in patients with papillary RCC, VEGF, VEGFR-1, and VEGFR-2 mRNA levels above the median were related to adverse survival. Immunohistochemical analysis has been performed with antibodies directed against VEGF-A, VEGF-C, VEGF-D, VEGFR-1, VEGFR-2, and VEGFR-3 on TMA constructed from paraffin-embedded clear cell and papillary RCC nephrectomy specimens to evaluate the role of the VEGF-VEGFR family in different histologic subtypes of RCC [208]. Protein expression was analyzed within the tumor epithelium, as well as in tumor-associated endothelium. This data may shed light on whether other signaling pathways aside from VHL may be promoting VEGF production and if so, consideration should be made for the inclusion of patients with histologic non-clear cell subtypes for therapies that target the VEGF ligands and/or their receptor tyrosine kinases. In addition, VEGF receptors were identified on the surface of RCC cells [208], suggesting that VEGF may augment RCC tumor growth through an autocrine loop. In addition, survival and metastatic pattern according to expression of VEGF-VEGFR family in clear cell RCC has been evaluated [209]. Analysis of these markers may allow for insight in predicting sites of metastatic spread, as well as providing additional markers for determining DSS.

#### 4.2.2. Microvascular density (MVD)

Microvascular density (MVD) is an often-quantified variable of tumor vasculature. The value of MVD as a predictor of prognosis in RCC is controversial. Several reports have shown a positive correlation between MVD and survival [210-214], whereas others have reported an inverse relationship [215-217.], others were unable to find a significant correlation [218,219]. A recent study has identified two distinct types of microvessels in clear cell RCC: undifferentiated (CD31+/CD34-) and differentiated (CD34+) vessels [220]. Higher undifferentiated MVD significantly correlated with higher tumor grades and shorter patient survival. Multivariate analyses showed that undifferentiated MVD was an independent prognostic factor for poor patient survival. Furthermore, an inverse correlation between undifferentiated MVD and differentiated MVD was identified in clear cell RCC.

#### 4.3 mTOR pathway

Another regulator of HIF1 $\alpha$  levels in the cell is mTOR, whose signaling activity acts to increase the cellular levels of HIF1 $\alpha$ , accentuating the overall elevation in levels caused by the absence of adequate pVHL function [221].

mTOR inhibitors have been previously described, and laboratory experiments have shown that antiproliferative effects of these inhibitors in RCC may result from the interruption of essential survival pathways and autophagy [222]. The effect of mTOR inhibitors on angiogenesis is likely to have an important function in RCC pathogenesis, a highly vascular tumor [222].

mTOR is a highly conserved serine/threonine kinase that forms quaternary complexes and has a key function in apoptosis, cell growth and tumor proliferation by controlling cellular catabolism and anabolism [222]. mTOR may complex with a regulatory-associated protein of mTOR to form mTORC1 and can also complex with a rapamycin-insensitive companion of mTOR, to form another multimolecular complex named mTORC2. (Rapamycin is an inhibitor of mTOR). mTORC1 may eventually be activated by growth factors and also the VEGF-R, PDGF-R, EGF-R, IGF receptor, and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathways [223]. At the molecular level, it is known that tumor angiogenesis depends on vascular growth factors such as VEGF, PDGF, bFGF and members of the TGF- $\beta$  superfamily. As these factors have been shown to be able to activate the PI3K/Akt/mTOR in cancer cells, endothelial cells or pericytes as described, mTOR complexes are also implicated in tumor angiogenesis biology [224].

Once activated, mTORC1 acts through its downstream effectors to stimulate protein synthesis, entrance into the G1 phase of the cell cycle and asserts control over proteins that regulate apoptosis. The witnessed activity of mTOR inhibitors in RCC has raised the possibility that patients who respond to this therapy share a common molecular phenotype that renders these tumors dependent on mTOR for growth and survival. It has been previously demonstrated that PI3K/Akt/mTOR signaling pathway inhibitors target tumor growth indirectly at the tumor level by interacting with the maintenance of endothelial cells and pericytes that are required for tumor angiogenesis [225].

A major stimulus of cancer angiogenesis is tissue hypoxia likely driven by tumorigenesis and growth initially lacking adequate blood supply. These conditions activate HIF1 $\alpha$ , and the mTOR pathway further enhances the translation of HIF1 $\alpha$  mRNA, thereby increasing the overall vasculogenic effect [226]. The observed clinical efficacy of mTOR inhibitors in RCC is mediated in part by dependence on efficient HIF translation in the mTOR pathway by interfering with the VEGF/VEGF-R and/or PDGF/PDGF-R signaling cascades. In summary, these data strongly suggest that the anticancer effects of mTOR inhibitors involve antiangiogenic processes mediated by effects on endothelial cells and pericytes, rather than on RCC themselves [221].

#### 4.4 NF- $\kappa$ B pathway

Nuclear Factor kappa B (NF- $\kappa$ B) is a family of transcription factors that has been associated with diverse cellular functions.

NF- $\kappa$ B activation is associated with increased proliferation, tissue invasion, angiogenesis, inhibition of apoptosis, and the development of drug resistance [227]. NF- $\kappa$ B activation has also been associated with proliferative responses mediated by induction of expression of cyclin D1, which drives the transition from the G1 to the S phase of the cell cycle [228]. VHL loss ultimately drives NF- $\kappa$ B activation by resulting in HIF $\alpha$  accumulation, which induces expression of transforming growth factor alpha (TGF- $\alpha$ ), with consequent activation of an EGF-R/phosphatidylinositol-3-OH kinase/protein kinase B (AKT)/I $\kappa$ B-kinase alpha/NF- $\kappa$ B signaling cascade [229].

#### 4.5 NO<sub>x</sub> pathway

Reactive oxygen species (ROS) regulate hypoxiaindependent and hypoxia-independent activation of HIF1 $\alpha$ . NAD(P)H oxidase systems are major sources of ROS. The NO<sub>x</sub> family of NAD(P)H oxidases have a core structure consisting of six transmembrane domains, including two hemebinding regions located at the N terminus and a cytoplasmic C terminus containing FAD- and NADPH-binding regions [230,231]. Reactive oxygen species, generated by NAD(P)H oxidases, are involved in signaling cascades of malignant growth. In VHL-deficient cells, NO<sub>x</sub>4 protein levels and NAD(P)H-dependent superoxide generation are increased. Reintroduction of VHL into the VHL-deficient cells down-regulates NADPHdependent superoxide generation [232].

#### 4.6 Tyrosin kinase pathway

Receptor tyrosine kinases (RTKs) constitute a superfamily of transmembrane proteins that relays signals from extracellular growth factors into the cell [233,234]. The TAM subfamily of RTKs contains the receptors Axl, Tyro3, and Mer [235,236]. They have in common a unique extracellular domain composed of two N-terminal immunoglobulin-like domains and two fibronectin type III repeats similar to the structure of neural cell adhesion molecules (NCAMs). TAM receptors share the same ligand, Gas6, a product of the growth arrest-specific gene 6 [237,238]. Gas6, cloned from serum-starved fibroblasts, is a member of the vitamin K-dependent family of Gla proteins homologous to the blood coagulation protein S [239]. Axl has been shown to affect neovascularization in vitro, and loss of Axl expression in tumor cells blocks growth of human neoplasms [240]. Perhaps, Axl on its own, by hemophilic interactions and by a kinase domainindependent mechanism [241], contribute to the disease-specific angiogenic programming



during VHL loss in tumor cells in parallel with angiogenic factors such as VEGF. Gas6 signaling via Axl, on the other hand, has been shown to have inhibitory effects on the VEGFR-driven angiogenic program [242]. Gas6-mediated activation of Axl in clear cell carcinoma cells results in Axl phosphorylation, receptor down-regulation, decreased cell-viability and migratory capacity [243].

#### 4.7 Mitogen-activated protein kinase pathway

Mitogen-activated protein kinase (MAPK) kinases (MKK) are crucial enzymes at the intersection of several biological pathways that regulate cell differentiation, proliferation, and survival. In response to a variety of extracellular stimuli, MKKs become activated and then phosphorylate MAPKs, including extracellular signalregulated protein kinase (ERK), c-Jun-NH2 kinase (JNK), and p38 MAPK (p38) [244,245] Overexpression of MKK has been described in human RCC cases [246]. Sustained activation of ERK has been established as a requirement for angiogenesis as well [247,248].

#### 4.8 HSP70 pathway

The major hsp70 are encoded by a duplicated locus (hsp70-1, hsp70-2) located in the MHC region, 92 kb telomeric to the C2 gene [249]. This segment of the MHC has been proposed to be termed the class IV region since it includes at least seven genes implicated to some degree in inflammation and in stress responses [250]. The two intronless genes (hsp70-1 and hsp70-2) encode an identical protein product of 641 amino acids. Hsp70-2 may have a potential role in cancer pathogenesis by participating in the regulation of antitumor immunity such as acting as a chaperone molecule for immunogenic tumor-associated peptides but also in regulatory processes such as the cell cycle. The possibility that a mutated hsp70-2 chaperone might have a dominant effect in tumor cells in triggering the G2/M phase transition during mitotic cell cycle cannot be excluded at the present time. Interestingly, among more than 100 RCC tumors studied in the laboratory, RCC-7 is the most aggressive one, with a rapid doubling interval in vitro and a high growth rate in SCID/nu mice. Preliminary experiments testing either the immortalizing or the transforming capacity of the mutated versus wild-type hsp70-2 cDNA in recipient cells have not led, however, to any direct evidence that the mutation plays a role in the oncogenic process [251].

#### 4.9. Carbonic anhydrase IX

Carbonic anhydrase IX (CA IX) is thought to play a role in the regulation of intracellular and extracellular pH during periods of hypoxia in tumor cells [252]. Overexpression of the CA IX gene has been demonstrated in RCC specimens [253,254]. CA IX is not expressed by normal fetal or adult kidney specimens, suggesting that it does not play a role in organogenesis, but rather is a product of tumor biology [255]. CA IX expression also varies among different kidney tumors and positive immunohistochemical staining has been shown in clear cell, granular, spindle, and papillary RCC, but is absent in chromophobe RCC and oncocytoma [256]. CA IX is located downstream of the von Hippel–Lindau (VHL) tumor suppressor protein and expression is regulated in part by hypoxia [257] through hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) (Figure 2) [258]. Loss of function of VHL can also lead to HIF-1 $\alpha$  accumulation [259], and up-regulation of CA IX expression. Restoration of VHL function in RCC cell lines has been shown to down-regulate CA IX expression to normal levels [260].

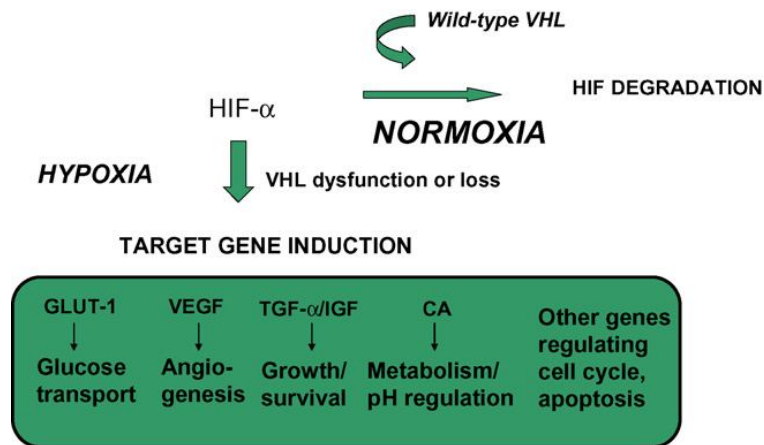


Figure 2

Hypoxia-inducible pathway and points of drug intervention currently being exploited. Oxygen homeostasis strictly modulates HIF-1 $\alpha$  transcription activation and HIF-1 $\alpha$  expression through VHL-mediated proteasomal protein degradation. The intracellular level of HIF protein plays an important role in regulating expression of an array of genes, which encode proteins essential to cancer cell functions under hypoxic conditions, such as glucose transport, angiogenesis, glycolysis, cell growth, migration and pH control. Effects of these proteins on hypoxic tumor cells are indicated. Signaling occurs through a variety of cell surface receptors including epidermal growth factor receptor (EGFR), HER2, vascular endothelial growth factor receptor (VEGFR) and type I insulin-like growth factor (IGF-I) receptor. The phosphatidylinositol 3'-kinase (PI3K)/AKT-1 and Ras/Raf/mitogen-activated protein/extracellular signal regulated kinase (MEK)/mitogen-activated protein kinase (MAPK) pathways converge through mTOR and HIF-1  $\alpha$ , respectively.

The first report of CA IX immunohistochemical staining in RCC found positive expression in 46 of 47 primary and 7 of 8 metastatic lesions [261]. Bui et al. [262] confirmed the high specificity of CA IX staining and reported 94% of clear cell RCC tumor samples to stain positively for CA IX. Recursive partitioning survival tree analysis determined that a cut-off of 85% CA IX staining provided the most accurate prediction of survival. Low CA IX staining was found to be an

independent prognostic indicator of poor survival in patients with metastatic RCC. CA IX significantly substratified patients with metastatic disease when analyzed by T-stage, grade, nodal involvement, and PS. For patients with non-metastatic RCC and at high risk for progression, low CA IX predicted a worse outcome similar to patients with metastatic disease. CA IX expression has also been combined with Ki67, a marker of cellular proliferation, to further stratify patients into low- (high CA IX/low Ki67), intermediate- (high Ki67 or low CA IX) and high-risk (high Ki67/low CA IX) groups to predict survival in RCC [263]. Furthermore, the combined parameter consisting of Ki67 and CA IX was a significant independent predictor of survival ( $p < 0.001$ ) and it was able to displace nuclear grade. CA IX expression in the peripheral blood has also been shown to be associated with decreased DFS in patients with renal cortical tumors [264].

CA IX expression has also been shown to predict for response to IL-2-based immunotherapy. Bui et al. [262] reported that all complete responders (8%) to IL-2-based regimens were patients with high CA IX (>85%) staining of the primary tumor [262]. Atkins et al. [265] explored this hypothesis and demonstrated that high CA IX expression was associated with IL-2 response. Using an identical cut-off of 85%, patients with high CA IX expression were twice as likely to have a response to treatment with IL-2 that resulted in prolonged survival. Thus, CA IX expression may be used to select a subset of patients that have a high likelihood of responding to treatment. These findings may also partly explain why patients with papillary and chromophobe subtypes, which express low levels or do not express CA IX, respond poorly to immunotherapy [105,266]. Although these findings are intriguing and have important prognostic implications for improving the selection of patients with metastatic RCC best suited for immunotherapy, this test still remains investigational and is currently used only in the context of clinical trials. Upton et al. [267] recently examined pathology from patients with RCC treated with IL-2 and reported that patients with non-clear cell RCC or with clear cell RCC with papillary, no alveolar, and/or more than 50% granular features respond poorly to IL-2.

#### 4.10. Hypoxia-inducible factor

Several series have examined VHL mutations in RCC tumors and resulting HIF- $\alpha$  expression [268-270]. Investigators showed a high percentage of HIF- $\alpha$  expression regardless of VHL mutation status, and an equal or higher percentage of HIF- $\alpha$  expression when analysis was restricted to the VHL-mutated subset. Non-clear cell RCC tumors were not found to have VHL mutations, and the resulting expression of HIF- $\alpha$  in 19 non-clear cell RCC tumors was low (24%; 6 of 25 tumors). HIF- $\alpha$  expression was not detected in normal renal tissue. The role of HIF-1 $\alpha$  in determining prognosis was recently examined in a cohort of 92 patients undergoing nephrectomy [271]. HIF-1 $\alpha$

was differentially expressed between histological subtypes, with clear cell displaying greater expression compared to either papillary or chromophobe subtypes. For clear cell RCC, increased HIF-1 $\alpha$  expression was a favorable independent predictor of survival. HIF-1 $\alpha$  expression in RCC was also recently analyzed using TMA [272]. In patients with clear cell RCC, HIF-1 $\alpha$  levels were significantly lower in locally aggressive tumors versus localized tumors, and patients with high HIF-1 $\alpha$  expression in the primary tumor demonstrated a trend towards prolonged survival.

#### 4.11. Immunomodulatory

##### 4.11.1 B7-H1

B7-H1 is a cell surface glycoprotein that acts as a T-cell costimulatory molecule that has been implicated as a potent negative regulator of antitumor immunity by inducing T-cell apoptosis, inhibiting cytokine production, and decreasing the cytotoxic effect of activated T-cells [273-275]. Aberrant expression of B7-H1 by tumor cells has been implicated in impairment of T-cell function and survival. Thompson et al. [276] showed that patients with high tumor and/or lymphocyte B7-H1 levels exhibited aggressive tumors and were 4.5 times more likely to die from their cancer than patients exhibiting low levels of B7-H1 expression. In addition, a high percentage of RCC metastases were shown to harbor B7-H1 [277], which may represent a promising therapeutic target. In an analysis of 306 patients who underwent nephrectomy for clear cell RCC, the 5-year DSS rates were 42% and 83% for patients with and without tumor B7-H1, respectively [278]. B7-H1 remained associated with disease-specific death even after adjusting for TNM stage, grade, and PS. In the subset of 268 patients with localized RCC, tumor B7-H1 was significantly associated with metastatic cancer progression and death from RCC even after adjusting for stage, grade, and PS.

##### 4.11.2. B7-H4

B7-H4 is a recently described B7 family coregulatory ligand that has been implicated as an inhibitor of T-cell-mediated immunity. Although expression of B7-H4 is typically limited to lymphoid cells, aberrant B7-H4 expression has also been reported in several human malignancies. Krambeck et al. [279] examined B7-H4 expression in 259 RCC specimens from patients treated with nephrectomy.

B7-H4 expression was associated with adverse clinical and pathologic features, including constitutional symptoms, tumor necrosis, and advanced tumor size, stage, and grade. Patients with tumors expressing B7-H4 were also three times more likely to die from RCC compared with patients lacking B7-H4. In addition, 211 (81.5%) specimens exhibited tumor vasculature endothelial

B7-H4 expression, whereas only 6.5% of normal adjacent renal tissue vessels exhibited endothelial B7-H4 staining.

#### 4.11.3. Mononuclear cell infiltration

The impact of mononuclear cell infiltration on RCC biology has been controversial. Webster et al. [280] reported that patients with clear cell RCC that had mononuclear cell infiltration within tumor were over 2 times more likely to die from RCC compared with patients whose specimens exhibited no mononuclear cell infiltration. Patients with specimens that had mononuclear cell infiltration exhibited a significantly increased likelihood of dying from RCC compared with patients whose specimens had no mononuclear cell infiltration even after adjusting for the Mayo Clinic SSIGN score.

#### 4.12. Proliferation

There have been a number of reports on the prognostic utility of molecular proliferation markers in RCC. Increased staining of proliferating cell nuclear antigen (PCNA) [281,282], Ki-67 [150, 282-284], and argyrophilic nucleolar organizer regions (Ag-NORs) [282,285-287] have all been shown to correlate with poor survival in small studies of patients with RCC. Visapaa et al. [284] correlated the expression of Ki-67 and gelsolin, an actin-binding protein, with grade, stage, and survival in patients with clear cell RCC using TMA. Stage was the most significant predictor of DSS, followed by Ki-67. Increased Ki-67 expression and decreased gelsolin expression in the same tumor was suggestive of poor DSS in patients with grade 2 tumors.

#### 4.13. Apoptosis

##### 4.13.1. p53

The tumor suppressor gene p53 has been coined the “guardian of the genome” as it plays a vital role in regulating the cell cycle and inducing apoptosis when DNA damage occurs [288]. Expression of p53 in RCC has been reported to be 16–57% [289-291]. Overexpression of p53 varies with histological subtype as is more frequently seen in papillary compared to chromophobe or clear cell RCC [292]. The role of p53 in prognosis was reviewed in an early series of 50 tumor specimens. In this small cohort, p53 overexpression was an independent predictor of poor survival [289]. Zingeuner et al. [290] analyzed 184 primary and 56 metastatic RCC specimens and reported that overexpression of p53 was more frequently seen in metastatic specimens and was found to be an independent prognostic marker for disease progression for clear cell RCC. Shvarts et al. [291]

reported on a TMA study containing tumors from 193 patients with non-metastatic RCC. Patients with localized tumors demonstrating mean p53 staining values above and below 20% of cells had recurrence rates of 37.7% and 14.4%, respectively. Furthermore, high expression of p53 was the strongest independent predictor of disease recurrence after nephrectomy, in addition to T-stage and ECOG-PS. Increased staining for p53 has also been correlated with worse survival in patients with clear cell RCC [293,294].

#### 4.13.2. Bcl-2

The bcl-2 gene encodes a survival protein that is known to inhibit of apoptosis. While its overexpression is thought to play an important role in tumorigenesis, its role in RCC is unclear. There are conflicted reports regarding expression of bcl-2 in clear cell RCC, ranging from 10 to 80% in published cohorts [292,295,296]. In a small cohort of 28 patients, there was a significant correlation between bcl-2 expression and higher tumor grade, but not with recurrence, metastasis, or overall survival. In a cohort of 101 patients with localized disease, bcl-2 positivity was correlated with improved survival and associated with lower stage and grade [296]. In patients with advanced RCC, bcl-2 expression has also been assessed. Lee et al. [295] published a cohort of patients with stage T3 clear cell RCC that underwent nephrectomy and resection of metastatic foci when indicated. The immunohistochemical pattern of bcl-2 expression was observed to be either expressed at low levels or absent and expression of bcl-2 did not predict DFS or DSS. RCC metastases displayed much greater expression of bcl-2 and could serve as a future target of therapy. However, uncertainty currently still exists regarding the prognostic role of bcl-2.

#### 4.13.3. Survivin

Survivin is an inhibitor of apoptosis that is expressed in a cell cycle dependent manner and is abundantly expressed in tumors of unfavorable histology [297]. Parker et al. [298] quantitated expression of survivin in 312 clear cell RCC specimens and analyzed the association of survivin expression with disease progression and cancer-specific survival. Patients who had high survivin expression levels were at significantly increased risk of death from RCC compared with patients who had low expression levels. The 5-year DSS rate was 43% for patients with high survivin expression and 87.2% for patients with low survivin expression. In multivariate analysis, survivin expression remained associated with death from RCC even after adjusting for the ECOG-PS, TNM stage, grade, and the Mayo Clinic SSIGN score. Among 273 patients who had localized clear cell RCC, survivin expression was significantly associated with cancer progression.

## 5 Treatment

At the present radical nephrectomy remains the most commonly performed standard surgical procedure for treatment of localized renal carcinoma, involves complete removal of Gerota's fascia and its contents, including a resection of kidney, perirenal fat, and ipsilateral adrenal gland, with or without ipsilateral lymph node dissection. Radical nephrectomy provides a better surgical margin than simple removal of the kidney, since perinephric fat may be involved in some patients. Twenty to thirty percent of patients with clinically localized disease develop metastatic disease after nephrectomy. Laparoscopic nephrectomy is a less invasive procedure than open radical nephrectomy, incurs less morbidity, and is associated with shorter recovery time and less blood loss. Laparoscopic partial nephrectomy can be considered at centers with experience in this procedure for early stage renal cell cancers generally less than 4 cm in largest dimension [299, 202]. Palliative nephrectomy should be considered in patients with metastatic disease for alleviation of symptoms such as pain, hemorrhage, malaise, hypercalcemia, erythrocytosis, or hypertension. Several randomized studies show improved overall survival in patients presenting with metastatic kidney cancer who have nephrectomy followed by either interferon or IL-2. If the patient has good physiological status, then nephrectomy should be performed prior to immunotherapy [300].

Nephron-sparing surgery (i.e., partial nephrectomy) has been shown to be equally as efficacious as radical nephrectomy, with reported local recurrence rates of < 2% and 5-year survival rates of 87-90%, which are comparable to those from radical nephrectomy [250]. This more limited treatment of a presumed cancerous lesion is generally reserved for lesions less than 4 cm in size along the largest dimension, for patients with poor renal reserve or function, or solitary kidney. In the case of a solitary kidney, though, there is increased risk of developing proteinuria, focal segmental glomerulosclerosis, and progressive renal failure exists if more than 50% of the renal mass is removed [301].

Micrometastasis to lymph nodes may be present in 10-25% of patients. The 5-year survival rate in patients with regional node involvement is substantially lower than in patients with stage I or II disease. Regional lymphadenectomy adds little in terms of operative time or risk and should be included in conjunction with radical nephrectomy [202,301-303]. Cryoablation and radiofrequency ablation, which may be undertaken laparoscopically or percutaneously, are promising techniques for treating small tumors [301,302].

## 5.1 Chemotherapy

A recent phase 2 trial of weekly intravenous gemcitabine (600 mg/m<sup>2</sup> on days 1, 8, and 15) with continuous infusion fluorouracil (150 mg/m<sup>2</sup>/day for 21 days in 28-day cycle) in patients with metastatic renal cell cancer produced a partial response rate of 17%. No complete responses were noted. Eighty percent of patients had multiple metastases, and 83% had received previous treatment. The mean progression-free survival duration of 28.7 weeks was significantly longer than that of historic control [303]. Floxuridine (5-fluoro 2'-deoxyuridine [FUDR]), 5-fluorouracil (5-FU), and vinblastine, paclitaxel (Taxol), carboplatin, ifosfamide, gemcitabine, and anthracycline (doxorubicin) have each been studied and used in the treatment of metastatic RCC. Floxuridine infusion has a mean response rate of 12%, while vinblastine infusion yielded an overall response rate of 7%. 5-FU alone has a response rate of 10%, but when used in combination with interferon, it had a 19% response rate in some studies [304].

RCC is refractory to most chemotherapeutic agents because of multidrug resistance mediated by p-glycoprotein normally present in all cells. This protein is responsible for efflux of drugs and other compounds deemed foreign to cells. Normal renal proximal tubules and renal cell carcinoma both express high levels of p-glycoprotein [305].

### ***Immunotherapy***

Until the past 8–10 years, immunotherapy (or cytokine therapy) with interleukin-2 (IL-2) and interferon- $\alpha$  (IFN- $\alpha$ ) was the major therapeutic option for patients with RCC. As that modality became more refined, it was observed that patients with clear cell RCC are the most likely to respond to immunotherapy with a proportion achieving durable complete responses [306-311]. In fact, approximately 60–70% of the 10–15% who are complete responders appear to be cured after long-term follow up [306,309]. High-dose IL-2 has consistently produced a 15-20% response rate, 6-8% complete remission rate, and approximately 5% cure rate, however, it is a fairly toxic regimen [312,313]. IFN- $\alpha$  has provided modest survival benefit, has a more favorable toxicity profile, and is more easily administered than IL-2. As a result, IFN- $\alpha$  has been adopted as the control arm in many clinical trials evaluating the merits and characteristics of novel agents.

### ***Targeted therapies***

Novel therapies for metastatic RCC have targeted downstream effects of von Hippel-Lindau (VHL) gene inactivation and the resulting upregulation of HIF target genes, notably VEGF and PDGF. Blocking VEGF activity, and therefore inhibiting the production of blood vessels, impairs tumor



growth and produces a state of reduced or stable disease. The mammalian target of rapamycin (mTOR) is a key mediator of tissue growth, proliferation and angiogenesis, and its inhibition can also lead to reduced growth and stabilization, particularly in growing tissues such as tumors [314]. RCC, a vascular tumor resistant to standard chemotherapy, has thus become a model for effectiveness of antiangiogenesis and mTOR inhibitory therapy. Additionally, VEGF has been shown to be immunosuppressive, and thus inhibiting its effect may also enhance antitumor immunity [315-317]. Seven drugs have been approved for the treatment of RCC within the past 8 years. Four agents directly inhibit the VEGF receptor (VEGFR) thus blocking angiogenesis, two agents inhibit mTOR, and one is an antibody that binds directly with VEGF and prevents it engaging with its receptor. All of these agents also have other targets, which explains some of the toxicities and possibly additive antitumor effects as well as activity in a variety of other malignancies. All have demonstrated improvement in progression-free survival (PFS) in patients with RCC, in randomized clinical trials. Additionally, the two groups, anti-VEGF and mTOR inhibitors, have class-specific toxicities that vary in intensity based on binding affinity, and on inpatient variability. These include, for the anti-VEGF agents, hypertension, fatigue, diarrhea and hand-foot syndrome, and for the mTOR inhibitors, mucositis, hyperglycemia, hyperlipidemia and rarely interstitial pneumonitis [311].

Currently, five targeted agents are in clinical use for metastatic RCC: sunitinib, sorafenib, temsirolimus, everolimus, and bevacizumab.

Sunitinib (Sutent, Pfizer) was the first antiangiogenesis agent approved for treatment of metastatic clear cell RCC, and RCC was its first approved indication. This agent inhibits VEGFR tyrosine kinase (VEGRF TKI) and platelet-derived growth factor receptor (PDGFR), which supports the pericytes of new blood vessels. It inhibits multiple other targets, of potential importance in other tumor types (Flt 3, RAF and others) [318]. This drug interacts with an adenosine triphosphate binding site in multiple tyrosine kinase domains and prevents autophosphorylation; this multiple tyrosine kinase inhibitor (MKI) has shown an objective response rate of 31% and a median progression-free survival of 11 months for sunitinib-treated patients [319,320]. It is considered the standard-of-care treatment for patients with advanced-, good and intermediate-risk conventional clear cell RCC.

Sorafenib (Nexavar, Bayer HealthCare), another MKI, is considered second-line therapy after cytokine failure [321].

Temsirolimus (Torisel, Wyeth), an inhibitor of mammalian target of rapamycin (mTOR), is considered appropriate for patients with poor-risk metastatic RCC, irrespective of histology [322,323].

Everolimus is an oral mTOR inhibitor that was approved for the treatment of RCC following progression on a VEGFR TKI, based on a randomized study comparing everolimus with placebo. Patients could have had more than one prior therapy to enroll in this trial. There was a statistically significant PFS benefit compared with placebo (4.9 months *versus* 1.9 months, HR 0.33) and patients who received placebo could be treated with everolimus upon progression [324,325].

Bevacizumab (Avastin, Genentech), a humanized antibody to VEGF, has shown promise in patients with good and intermediate-risk metastatic RCC [326,327] but has not yet been approved for metastatic RCC by the U.S. Food and Drug Administration.

Bevacizumab (+ interferon-alpha), sunitinib, and temsirolimus (in poor-risk groups) have proven to be effective as first-line palliative treatments. Sorafenib has demonstrated benefits in patients that have failed prior therapy, as has everolimus after failure of sorafenib and/or sunitinib [328].

### ***Neoadjuvant Therapy and the Role of Cytoreductive Nephrectomy in the Era of Targeted Therapy***

While agents targeting the VEGF and mTOR pathways have an established role in the management of mRCC, we are yet to understand how these agents can be integrated with surgical approaches to maximize clinical benefit. Several studies have explored the feasibility and utility of VEGF pathway inhibitors in the neoadjuvant setting. The theoretical advantages of administering systemic therapy before surgery are many and include assessment of primary tumor response, tumor downstaging, and decreasing circulating tumor cells [329-331]. Early reports suggest that these therapies lead to modest regression of primary tumors, with sufficient downstaging in some patients to permit surgical extirpation of previously unresectable tumors [332,333]. Although studies have demonstrated the general tolerability of targeted agents, there is still limited data on the safety of surgical resection following treatment with these agents, and several reports have shown increased perioperative complications after treatment with sunitinib, sorafenib, or bevacizumab [334,335].

The concept of neoadjuvant therapy is attractive on many fronts; however, the approach must be validated in randomized clinical trials before being widely adopted. The role of cytoreductive nephrectomy in patients with metastatic disease receiving VEGF targeted agents is another area that deserves further study. The current paradigm of debulking nephrectomy is based on data generated in the era of cytokine therapy, but is commonly used as a prelude to targeted therapy.

Several ongoing studies are designed to clarify these critical issues. The SURTIME study (NCT01099423) from the EORTC is a phase III trial of 458 subjects with their primary tumor in situ and synchronous mRCC. Subjects are randomized to sunitinib followed by nephrectomy or nephrectomy followed by sunitinib with PFS as the primary endpoint. A second study, CARMENA

(NCT00930033), is a randomized phase III trial comparing sunitinib therapy alone versus cytoreductive nephrectomy followed by sunitinib therapy.

### ***Adjuvant Therapy for Clear Cell RCC***

A proportion of patients with seemingly organ confined and locally advanced RCC are at risk for progression after surgical resection. Adjuvant therapy has been used in the treatment of several malignancies with favorable results; however, cytokine treatment regimens, radiation therapy, and thalidomide have failed to improve outcomes in RCC when used in the adjuvant setting [336-338]. Several randomized, phase III trials are currently evaluating the impact of VEGF pathway antagonists on PFS, OS and safety/tolerability in the adjuvant setting.

The S-TRAC trial (NCT00375674) is a randomized, two arm, double-blind trial started in 2007. Subjects at high risk of recurrence based on the University of California Los Angeles Integrated Staging System (UISS) are randomized to receive sunitinib versus placebo for 12 months.

The ASSURE trial (NCT00326898) is a large double blind trial with an accrual goal of close to 2000 patients. Subjects with pT1b, G3-4, pT2-T4, or any T with node positive RCC are randomized after nephrectomy to receive 1 year of sunitinib, sorafenib, or placebo. The primary endpoint is disease free survival and the anticipated completion date for data analysis is 2016.

The EVEREST trial (NCT01120249) is a randomized, two arm, double-blind trial started in 2011, evaluating everolimus *versus* placebo therapy for 1 year in patients with either intermediate high-risk or very high-risk disease. The primary endpoint is recurrence-free survival.

The PROTECT trial (NCT01575548) is a randomized, double-blind study started in 2010, evaluating if administration of using pazopanib *versus* placebo can prevent or delay recurrence of kidney cancer in patients with moderately high or high risk (pT2, G3-4, pT3-T4, or any T with node positive RCC) of developing recurrence after undergoing kidney cancer surgery.

The last ongoing trial is the SORCE trial (NCT00492258) which is a three arm phase III study that randomizes subjects at high or intermediate risk of relapse using the Leibovich score to placebo for 3 years versus placebo for 2 years combined with sorafenib for 1 year versus sorafenib for 3 years. The trial's estimated primary completion date is in late 2012. The results of these trials are eagerly awaited to determine the role of targeted therapy in the adjuvant setting.

## Aim of the study

An important key point of previous studies is represented by the criteria used to select patients suitable for randomization. The wide variability of the inclusion criteria used to enroll patients can affect the sample size and consequently the number of expected events according to the planned primary endpoint. Indeed, to enroll patients with a low or intermediate risk of progression can increase the number of patients needed to be randomized to demonstrate eventually survival benefits between treated patients and placebo group.

The identification of predictors of disease-free survival (DFS) is important to improve the definition of selection criteria for new adjuvant studies. Several studies have assessed the role of molecular and cytogenetic markers, especially in patients with ccRCC, with the intent of increasing the prognostic accuracy achievable with the classic clinical and pathologic features [339-343,161,164,131,165,172].

The purpose of the present study was 1) to estimate the number of patients potentially suitable for ongoing adjuvant trials and 2) to test the appropriateness of the eligibility criteria used to recruit participants for clinical trials on adjuvant medical therapy considering the relative number of observed events in a large series of non-metastatic patients with renal cell carcinoma (RCC) treated with radical or partial nephrectomy in 16 academic centers in Italy and 3) to test whether the simultaneous presence of loss of chromosomes 9p and 14q was associated with a different risk of recurrence in the subgroup of patients suitable for adjuvant therapy with targeted therapies.

## Patients and methods

We queried a database generated in 2008 in the context of the Surveillance and Treatment Update Renal Neoplasms (SATURN) project promoted by the Leading Urological No profit foundation for Advanced (LUNA) research of the SIU (Italian Society of Urology). Specifically, a total of 16 academic centers in Italy provided clinical records of 5893 patients who underwent radical nephrectomy (RN) or partial nephrectomy (PN) between 1995 and 2007 because of a suspicion of kidney cancer. The patients with benign histology (n = 430) were excluded from the study. The 5463 remaining patients were the subjects of the present analysis. Surgery was performed by several surgeons according to the standard criteria for RN (ie, extrafascial dissection of the kidney). The hilar and regional lymph nodes adjacent to the ipsilateral great vessel generally were resected, along with enlarged lymph nodes if they were abnormal on preoperative CT scans or palpable intraoperatively. Extended lymphadenectomy was routinely performed in a few centers. In patients with a contralateral normal kidney, elective PN had been routinely indicated in the presence of single peripheral tumors  $\leq 4$  cm, although some referral centers also performed elective PN in the case of larger tumors. According to the purpose of the present study, we extracted from the database only patients with non metastatic (M0) RCC at the diagnosis. Therefore, patients who underwent cytoreductive nephrectomy or patients with non-clear cell RCC were excluded from the analysis. Preoperatively, all patients performed at least abdominal computed tomography (CT) scans and chest X-rays. Bone scans and brain CT scans were performed only when indicated by signs and symptoms. Table 1 summarizes the clinical and pathologic features of the 5463 analyzed patients.

**Table 1** – *Clinical and pathologic features of the 5463 analyzed patients*

<b>Variables</b>	
Age, yr, median (IQR)	63 (54–71)
Gender, No. (%)	
Male	3620 (66)
Female	1843 (34)
Mode of presentation, No. (%) *	
Incidental	3413 (62)
Local symptoms	1471 (27)
Systemic symptoms	266 (5)
Type of surgery, No. (%)	

Radical nephrectomy	3887 (71)
Elective partial nephrectomy	1341 (25)
Imperative partial nephrectomy	235 (4)
Histologic subtype, No. (%)	
Clear cell	4435 (81)
Papillary	590 (11)
Chromophobe	298 (6)
Collecting duct	48 (1)
Unclassified	92 (2)
Pathologic tumor size, median (IQR)	
	5 (3.5–7)
Pathologic T stage (TNM 2009), No. (%)	
T1a	1941 (35.5)
T1b	1486 (27)
T2a	447 (8)
T2b	156 (3)
T3a	1084 (20)
T3b	120 (2)
T3c	27 (0,5)
T4	202 (4)
Pathologic N stage, No. (%)	
Nx	3133 (57)
N0	2081 (38)
N1	119 (2)
N2	130 (2)
M stage, No. (%)	
M0	5133 (94)
M1	330 (6)
Fuhrman nuclear grade, No. (%)**	
G1	635 (12)
G2	2848 (52)
G3	1435 (26)
G4	417 (8)
*Missing in 313 (6%) ** Missing in 128 (2%) IQR = interquartile range.	

The following pathological variables were assessed: local extension and dimension of primary tumor, regional lymph nodes involvement, Fuhrman's nuclear grade and coagulative necrosis. All the included cases were reclassified according the eligibility criteria of each ongoing RCT testing adjuvant targeted therapies. For each category we calculated the number of observed events during

the follow-up and the progression-free survivals. Events were defined as patients who developed distant metastases during the follow-up period.

Patients were generally observed every 3 to 4 months for the first year after surgery, every 6 months from the second through the fifth years, and annually thereafter. Follow-up consisted of a history, a physical examination, routine blood work and serum chemistry studies, chest radiography, and radiographic evaluation of the controlateral or remnant kidney. Elective bone scan, chest CT, and magnetic resonance imaging (MRI) were performed when clinically indicated.

Finally, we evaluated the loss of chromosomes 9p and 14q in 175 patients who underwent PN or RN between 1990 and 2000 for non-metastatic ccRCC.

For each patient, we extracted the following variables: Eastern Cooperative Oncology Group performance status, pathologic tumor size, extension of primary tumor (pT), lymph node involvement (pN), presence of distant metastases (M), nuclear grade, and coagulative necrosis. Pathology slides were reviewed by a single uropathologist who had experience dealing with RCC. All cases included in the study were ccRCC. Pathologic stage was reassigned according to the 2002 version of the TNM [344]. Fuhrman classification [98] and the Mayo Clinic criteria [39] were used to reassign nuclear grading and coagulative necrosis. The pathologist was blinded to both original pathologic diagnoses and follow-up data.

All patients with synchronous metastasis were excluded from the analysis. All cases without metastasis at diagnosis were reclassified according to Leibovich score and UISS to estimate the risk of recurrence during follow-up. Specifically, Leibovich score stratifies three different categories: low risk (score 0–2), intermediate risk (score 3–5), and high risk (score >5) [161]. UISS subdivides patients without distant metastasis in three different categories: low, intermediate, and high risk [165]. Table 2 summarizes the clinical and pathologic features of the 175 nonmetastatic patients included in analysis (Table 2).

**Table 2:** *Clinical and pathological characteristics of 175 analyzed patients*

<b>Variable</b>	
Mean ± SD of age (years)	60.8±11.6
Gender (%)	
- male	128 (73%)
- female	47 (27%)
Symptoms (%)	
- absent	97 (55.4%)
- present	78 (44.6%)

Surgical treatment (%)	
- radical nephrectomy	144 (82.3%)
- partial nephrectomy	31 (17.7%)
TNM, 2002	
- pT1a	48 (27.4%)
- pT1b	50 (28.6%)
- pT2	26 (14.9%)
- pT3a	14 (8%)
- pT3b-c	35 (20%)
- pT4	2 (1.1%)
Lymph node involvement (%)	
- pNx/pN0	170 (97.1%)
- pN1-2	5 (2.9%)
Fuhrman Nuclear Grade (%)	
- grade 1	20 (11.4%)
- grade 2	59 (33.7%)
- grade 3	70 (40%)
- grade 4	26 (14.9%)
Coagulative tumor necrosis (%)	
- absent	153 (87.4%)
- present	22 (12.6%)
Leibovich score	
- low risk	70 (40%)
- intermediate risk	68 (39%)
- high risk	37 (21%)
UISS	
- low risk	54 (30.9%)
- intermediate risk	110 (62.9%)
- high risk	11 (6.3%)

### *Tissue microarray construction*

The tissue microarrays were constructed using a tissue arraying instrument (Beecher Instruments, Hackensack, NJ, USA). Tissue cylinders with a diameter of 0.6 mm were punched from each donor paraffin block in targeted areas corresponding to previously demarcated areas on the parallel hematoxylin and eosin-stained slide. Sections 5- $\mu$ m thick were cut from the master block, stained with hematoxylin and eosin and used for in situ hybridization. Three neoplastic cores were available for neoplastic and two for normal renal parenchyma adjacent to neoplastic tissue for each case.

### *Cytogenetic analysis*

We performed an interphase cytogenetic fluorescence in situ hybridization (FISH) analysis using a telomeric-specific probe (115 kb) mapping on the chromosome 9p and 14q telomeres (SpectrumGreen LSI, Abbott) and a centromeric (alpha-satellite DNA) probe mapping on



chromosome 9p11-q11. Five-micrometer sections were cut from paraffin-embedded blocks, and the FISH analysis was performed [341]. Each probe was diluted 1:100 in tDenHyb2 buffer (Insitus, Albuquerque, NM, USA). The slides were examined using an Olympus BX-61 (Germany) with appropriate filters for SpectrumOrange (Tel 9p, Tel 14q; Vysis-Abbott), SpectrumGreen (centromeric probe 9; Vysis-Abbott), and the UV filter for the 4',6-diamidino-2-phenylindole nuclear counterstain. The signals were recorded with a D-Sight Fluo (Menarini Diagnostic). Fluorescent in situ signals were evaluated in accordance with previous reports [341]. Briefly, for centromeric chromosome 9 and telomeric chromosomes 9p and 14q, percentages of single signals >28%, >32%, and >34%, respectively, were considered to indicate chromosomal loss.

### *Statistical analysis*

Parametric continuous variables were reported as mean value plus or minus standard deviation, whereas median value and interquartile range (IQR) were used for the nonparametric continuous variables. DFS was calculated from the date of nephrectomy to the date of distant recurrence or last contact. Distant metastases were determined either clinically by radiologic imaging technique or histologically by biopsy of metastatic sites. The Kaplan-Meier method was used to calculate survival functions, and differences were assessed using the log rank test. Patients with disease progression were considered as events. Statistical significance in this study was set as  $p < 0.05$ . All reported  $p$  values are two-sided. Analyses were performed with SPSS vers. 21.0 (SPSS Inc, Chicago, IL, USA).

Patients who were alive and disease free or who had died from other causes were censored. The log-rank test was used to compare survival curves. Univariable and multivariable Cox regression models addressed time to recurrence after surgery. We generated different multivariable models with the intent of demonstrating the independent predictive role of cytogenetic abnormalities once adjusted for the effects of the most common tools (UISS, Leibovich score, pT, and nuclear grade) used to stratify patients in the ongoing phase 3 trials evaluating the efficacy of adjuvant therapies. Statistical analyses were performed using the SPSS software package v.16.0, with two-sided  $p$  values  $<0.05$  considered statistically significant.

## Results

We analyzed 5,194 patients with non-metastatic CCRCC at diagnosis. The median follow-up was 42 mo (IQR 24–75). Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma (ASSURE) and Everolimus in Treating Patients With Kidney Cancer Who Have Undergone Surgery (EVEREST) trials used the same criteria to select patients suitable for randomization. Specifically, all cases with pT1bG3-4; pT2 any G and pT3-4 any G RCC can be enrolled to receive active treatment or placebo. Specifically, according to our database, 2,090 (41%) cases reached previous criteria. In details, 406 (19.5%) were pT1bG3-4; 565 (27%) were pT2 any G and 1,141 (54.5%) were pT3-4 any G. The number of observed events was 53 (13%); 119 (21%) and 466 (41%), respectively. PROTECT trial randomized patients with pT2G3-4 or pT3-4 any G RCC to receive pazopanib or placebo. In our database, an overall number of 1360 (27%) cases meet previous selection criteria. Specifically, 219 (16%) pT2G3-4 and 1141 (84%) pT3-4 any G. The observed events were 59 (27%) in the first subgroup and 466 (41%) in the second one.

The S-TRAC trial used the UCLA Integrated Staging System (UISS) to select patients suitable to be randomized in Sunitinib or Placebo arms. In details, only patients with high risk (pT3-4, grade 2-4, performance status ECOG >0) or very high risk RCC (pN1, any G any PS) were randomized. In our database information concerning UISS was present only in 2933 patients because of performance status was not available in all cases. Overall 352 (12%) of patients meet the selection criteria: 243 (69%) high risk and 109 (31%) very high risk. We observed 87 events (36%) in the first subgroup and 74 (68%) events in the second one. Finally, selection criteria used in the SORCE trial were based on the Leibovich criteria. Specifically, patients with intermediate or high risk of progression were randomized to receive Sorafenib or Placebo after nephrectomy. Leibovich score was assigned in a total of 3,137 patients included in our database. In particular, 1088 (35%) were intermediate risk and 261 (8%) were high risk. The number of observed events was 209 (20%) and 137 (53%), respectively. (Tables 3 and 4). (Figures 3,4 and 5)

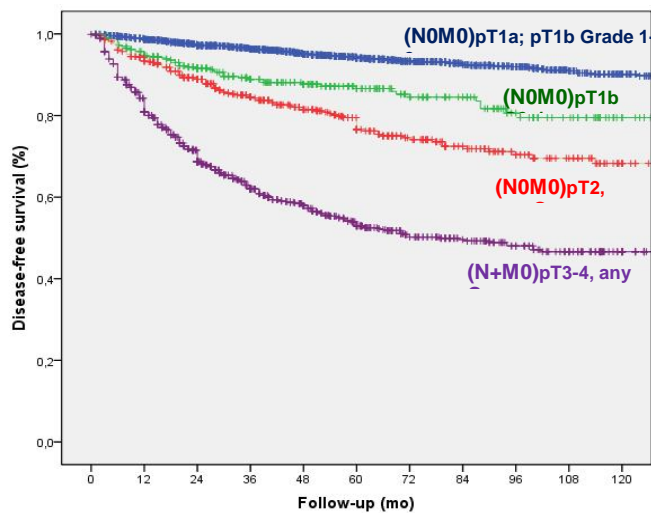
**Table 3:** Summary of ongoing RCTs testing the adjuvant role of targeted therapies in the treatment of non metastatic renal cell carcinoma (RCC).

<b>Trial</b>	<b>Treatment</b>	<b>Primary outcome</b>	<b>Histologic subtypes</b>	<b>Stratification tools</b>
<b>ASSURE</b>	Sunitinib vs Sorafenib vs Placebo	PFS	Clear cell & non-clear cell	pT, Grading
<b>S-TRAC</b>	Sunitinib vs Placebo	PFS	Clear cell & non-clear cell	UISS
<b>SORCE<sup>9</sup></b>	Sorafenib vs Placebo	PFS	Clear cell & non-clear cell	Leibovich score
<b>EVEREST</b>	Everolimus vs Placebo	PFS	Clear cell & non-clear cell	pT Grading
<b>PROTECT</b>	Pazopanib vs Placebo	PFS	Prominent clear cell	pT, Grading

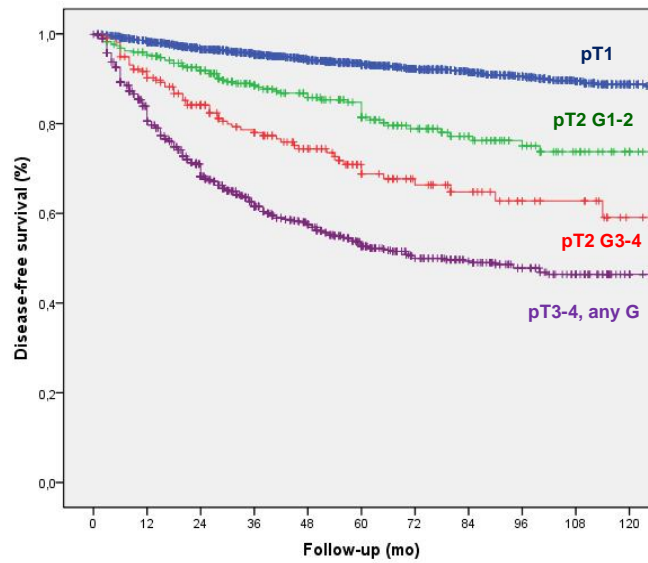
**Table 4:** Summary of results of present study

Trial	Number of patients enrollable (%)	Selection criteria (%)	Observed events (%)	Ideal categories (% of events)
<b>ASSURE EVEREST</b>	2,090 (41%)	pT1bG3-4: 406 (19.5%) pT2 any G: 565 (27%) pT3-4 any G: 1141 (54.5%)	638 (30.5%)	pT3-4 any G (41%)
<b>PROTECT</b>	1,360 (27%)	pT2 G3-4: 219 (16%) pT3-4 any G: 1141 (84%)	525 (39%)	pT3-4 any G (41%)
<b>S-TRAC</b>	352 (12%)	High risk: 243 (69%) Very high risk: 109 (31%)	161 (45.7%)	High risk and very high risk (45.7%)
<b>SORCE</b>	1349 (43%)	Intermediate risk: 1088 (35%) High risk: 261 (8%)	346 (25.6%)	High risk (53%)

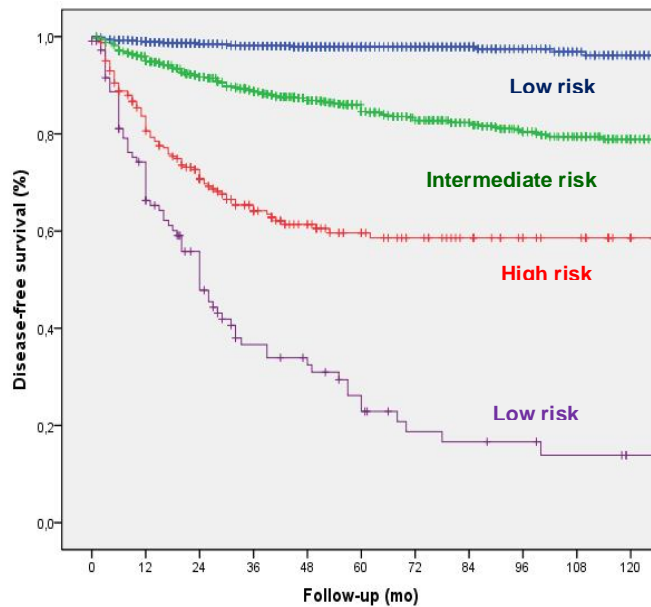
**Figure 3:** Progression-free survival according to selection criteria used in the context of ASSURE and EVEREST trials.



**Figure 4:** Progression-free survival according to selection criteria used in PROTECT trial



**Figure 5:** Progression free survival according to UCLA Integrated Staging System. Selection criteria used in the context of S-TRAC trial



Concerning the cytogenetic analyses performed in a small subgroup of cases, twenty-one patients (10.7%) with distant metastases at diagnosis were excluded from the analysis.

No cytogenetic abnormalities were observed in 135 cases (77.1%), and loss of chromosome 9p or 14q was detected in 14 cases (8%) and 9 cases (5.1%), respectively. The contemporary presence of both cytogenetic alterations was reported in 17 cases (9.7%).

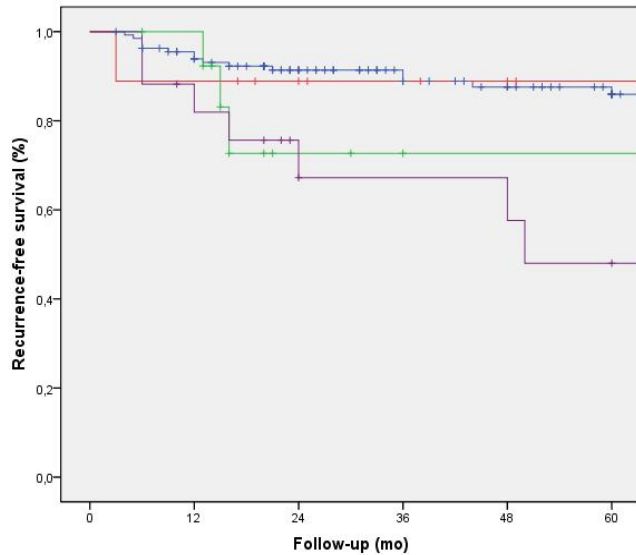
The median follow-up duration was 36 mo (IQR: 21–78). At follow-up, 136 patients (77.7%) were alive and disease-free, 6 (3.4%) were alive with disease recurrence, 7 (4%) had died of other causes, and 26 had died of RCC. Median follow-up of the 136 living patients was 44 mo (IQR: 25–80). The 5- and 10-yr DFS estimates were 81% (standard error [SE]: 0.3%) and 69% (SE: 0.6%), respectively. Table 5 summarizes the results of univariable Cox regression analyses (Table 5).

**Table 5:** *Univariable analysis predicting recurrence-free survival*

Variables	HR	95% CI	P value
Size (continuous)	3.421	1.199 - 9.759	0.02
pT (2002)			0.001
- pT1a	1	referent	
- pT1b	4.560	0.532-39.055	0.16
- pT2	13.089	1.610-106.636	0.01
- pT3a	32.921	4.033-268.757	0.001
- pT3b-c	20.129	2.594-156.223	0.004
- pT4	19.269	1.189-312.259	0.03
pN (2002)			
- pN0/Nx	1	referent	
- pN1-2	4.243	1.284-14.022	0.10
Nuclear Grade			0.001
- Grade 1-2	1	referent	
- Grade 3	1.653	0.675–4.048	0.27
- Grade 4	6.620	2.692 – 16.280	0.001
Coagulative necrosis			
- absent	1	referent	
- present	4.791	2.339 – 9.814	<0.001
Leibovich score			<0.001

- low risk (0-2)	1	referent	
- intermediate risk (3-5)	3.247	1.045 - 10.088	0.04
- high risk (>5)	10.492	3.477 – 31.280	<0.001
UISS			0.01
- low risk	1	referent	
- intermediate risk	4.284	1.292 – 14.206	0.01
- high risk	8.946	1.992 – 40.177	0.004
Cytogenetic abnormalities			0.01
- absent	1	referent	
- loss 9p	2.661	0.902-7.856	0.07
- loss 14q	1.060	0.141-7.969	0.95
- loss 9p and 14q	3.877	1.604-9.376	0.003

Pathologic extension of primary tumor ( $p < 0.001$ ), lymph node involvement ( $p = 0.01$ ), nuclear grade ( $p < 0.01$ ), coagulative tumor necrosis ( $p < 0.001$ ), tumor size ( $p = 0.02$ ), Leibovich score ( $p < 0.0001$ ) and UISS ( $p < 0.0001$ ) were all associated with DFS in univariable analysis. Specifically, according to the Leibovich score, the 5-yr DFS estimates were 96.8%, 76.3%, and 54.4% in the low-, intermediate-, and high-risk groups, respectively (log-rank,  $p < 0.001$ ). According to UISS, the 5-yr DFS estimates were 95.8%, 77.4%, and 36.4% in the low-, intermediate-, and high-risk groups, respectively (log-rank,  $p = 0.006$ ). Figure 6 shows the DFS according to the different cytogenetic abnormalities evaluated (Fig. 6).



**Figure 6:** Recurrence-free survival stratified according to absence or presence of the evaluated cytogenetic abnormalities. The DFS of patients with loss of 9p (green line) was overlapping to patients without cytogenetic abnormalities (blue line) (log rank  $p$  value 0.07). The DFS of patients with loss of 14q (red line) was similar to those of patients without alterations (log rank  $p$  value 0.96). The contemporary presence of loss 9p and loss 14q (violet line) was associated with a significant worse DFS in comparison with normal cases (log rank  $p$  value 0.001)

Patients with loss of only chromosome 9p (log-rank,  $p = 0.07$ ) or only chromosome 14q (log-rank,  $p = 0.96$ ) showed DFS similar to patients without these cytogenetic abnormalities. Conversely, cases with the contemporary presence of both abnormalities showed a significantly lower DFS in comparison with normal cases (log-rank,  $p = 0.001$ ).

Table 6 summarizes the results of multivariable Cox regression analyses (Table 6).

**Table 6:** Multivariable Cox regression models predicting recurrence-free survival: model 1 including pT and nuclear grading; model 2 including Leibovich score and model 3 including UISS

	Variables	HR	95% CI	P value
Model 1	pT			0.01
	- pT1a	1	referent	
	- pT1b	5.241	0.605 - 45.376	0.13
	- pT2	18.081	2.155 - 151.677	0.008
	- pT3	23.668	2.850 - 196.536	0.003
	- pT4	15.559	1.921 - 126.005	0.01
	Grading			
	- Grade 1-2	1	referent	
	- Grade 3-4	3.215	1.146 - 9.023	0.02
Loss 9p and 14q				
- Absent	1	referent		
- Present	4.479	1.767 - 11.868	0.002	
Model 2	Leibovich score			<0.001



	- Low risk (0-2)	1	referent	
	- Intermediate risk (3-5)	3.173	1.021 – 9.864	0.04
	- High risk (>5)	10.706	3.568 – 32.123	<0.001
	Loss 9p and 14q			
	- absent	1	referent	
	- present	3.704	1.565 - 8.768	0.003
Model 3	UISS			0.02
	- Low risk	1	referent	
	- Intermediate risk	4.176	1.259 – 13.856	0.01
	- High risk	8.110	1.801 – 36.522	0.006
	Loss 9p and 14q			
	- absent	1	referent	
	- present	3.194	1.351 – 7.553	0.008

The simultaneous loss of both chromosomes 9p and 14q turned out to be an independent predictor of DFS, once adjusted for the effects of pT and nuclear grade (HR: 4.579; 95% CI, 1.767–11.868), Leibovich score (HR: 3.704; 95% CI, 1.565–8.768) or UISS (HR: 3.194; 95% CI, 1.351–7.553) (Table 5). Specifically, contemporary loss of chromosomes 9p and 14q was able to further stratify patients included in the intermediate risk groups according to both Leibovich score (log-rank,  $p = 0.004$ ) and UISS (log-rank,  $p = 0.003$ ). A non–statistically significant trend was also observed in patients with loss of chromosomes 9p and 14q who were included in the high-risk group based on Leibovich score (log-rank,  $p = 0.07$ ). Non–statistically significant differences were observed in the high-risk group according to UISS (log-rank,  $p = 0.76$ ).

The contemporary presence of both cytogenetic alterations was reported in 17 cases (9.7%). The median follow-up duration was 36 mo (interquartile range: 21–78). The simultaneous loss of both chromosomes 9p and 14q turned out to be an independent predictor of DFS, once adjusted for the effects of pT and nuclear grade (hazard ratio [HR]: 4.579; 95% confidence interval [CI], 1.767–11.868), Leibovich score (HR: 3.704; 95% CI, 1.565–8.768), or UCLA Integrated Staging System (UISS; HR: 3.194; 95% CI, 1.351–7.553).

## Conclusions

Interpretation of ongoing RCTs testing adjuvant effect of targeted therapies in patient who underwent radical or partial nephrectomy for non-metastatic RCC could be strongly influenced by the used selection criteria. Indeed, our study clearly demonstrated that some trials (ASSURE, EVEREST and SORCE) used very large selection criteria randomizing also categories with a very limited risk of progression influencing significantly the number of events needed to demonstrated a statistically significant differences between treatment arms and placebo ones. Moreover, also the performance of the remaining two RCTs (PROTECT and S-TRAC) could be influenced by the inclusion of patients with localized disease (pT1b-2). Indeed, the ideal candidate for adjuvant trial seems to be patients with pT3-4 tumors regardless to Fuhrman nuclear grading.

Moreover, loss of chromosomes 9p and 14q was an independent predictor of DFS in patients who underwent PN or RN for nonmetastatic ccRCC, once adjusted for the effects of either Leibovich score or UISS, demonstrating that the recurrence-free survival of patients suitable for adjuvant protocols could be strongly influenced by the cytogenetic characteristics of the tumor.

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