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Prognostic Factors in Renal Cell Carcinoma: An Evaluation of T-Stage, Histopathological Grade, p53, Ki-67, COX-2, and Her-2 Expressions

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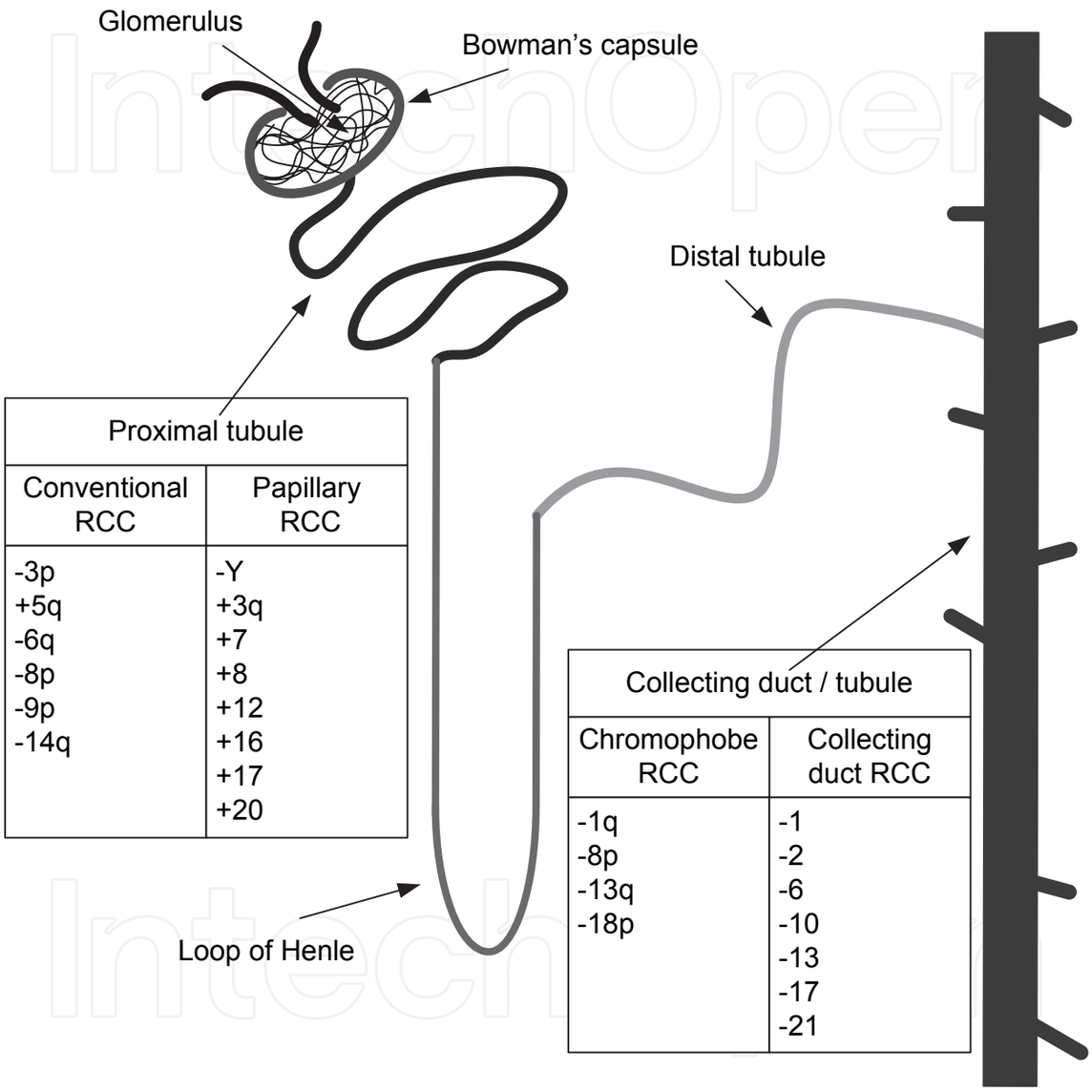
1. Introduction

Kidney cancer[1] represents 2-3% of all diagnosed malignancies worldwide although in some Northern and Central European countries the incidence is higher, even 4-5% (Ferlay, 2010). Kidney cancer is responsible for approximately 116,000 deaths per year worldwide (Ferlay, 2010). In the European Union (EU), the annual number of new kidney cancers was 73 171 in 2008 (Ferlay, 2010). The majority of renal cell carcinomas (RCCs) arise from the cells of renal proximal tubules of nephrons, but 5% of cases from the cells of the collecting ducts (Chao *et al.* 2002, Kovacs *et al.* 1997, Störkel *et al.* 1997) (Figure 1.). Renal tumors are members of a complex family with unique histology, cytogenetic defects and variable metastatic potential (Linehan *et al.* 2003, Thoenes *et al.* 1986). Of all RCCs, 70-80% is of conventional type, also known as clear cell RCCs. Of these, approximately 75% have a mutation in the von Hippel-Lindau tumor suppressor gene (*VHL),* in the short arm of chromosome 3 (Maxwell *et al.* 1999, Gnarra *et al.* 1994).

The annual increase in RCC incidence has been 2-4% since the 1970s (Finnish Cancer Registry 2007, American Cancer Society 2004, Mathew *et al.* 2002). This has been attributed to the use of radiological imaging which is able to find presymptomatic RCC lesions (Jayson and Sanders 1998), as well as the increased prevalence of etiologic risk factors, such as obesity (Chow *et al.* 2000) and cigarette smoking (Hunt *et al.* 2005). The increase has been highest in localized disease, especially in tumors with less than 4 cm in diameter (Hollingsworth *et al.* 2006). 30-60% of RCC tumors are found incidentally in abdominal imaging performed for some other reason than suspected renal tumor, such as the evaluation of non-specific musculoskeletal or abdominal complaints (Jayson and Sanders 1998). Macroscopic hematuria, palpable tumor and pain, together called the classic triad in RCC, indicate metastatic disease (Cunningham 1938). Metastatic disease is seen in 20-30% of RCC patients at diagnosis (Janzen *et al.* 2003, Mc

[1] In epidemiological statistics, RCC and renal pelvis cancer are usually not reported separately, but combined under the heading of kidney cancer (Parkin *et al.* 2003).

Nichols *et al.* 1981). Half of the patients diagnosed with local RCC will later have a recurrence of their cancer: two thirds within the first year (Janzen *et al.* 2003), and the majority within five years (Lam *et al.* 2005, McNichols *et al.* 1981). The risk for late recurrence, at over 10 years from nephrectomy, is at least 10% (McNichols *et al.* 1981).



The number reflects the chromosome in which its genetic aberration is located.

- means loss of function.
+ means gain of function.
p is the short arm of the chromosome.
q is the long arm of the chromosome.

Fig. 1. The genetic changes that characterize the different RCC subtypes according to the Heidelberg classification (Modified from Bodmer *et al.* 2002).

For those RCC patients with performance status enabling current treatments the expected five-year survival rate is slightly higher than 60% (Parkin *et al.* 2003). According to a few previous studies on long-term outcome for metastatic RCC (mRCC), the five-year survival is from 3% to 16% (Atzpodien *et al.* 2002, Motzer *et al.* 2000, Minasian *et al.* 1993) if metastasectomy has not been a possible treatment. For localized RCC, nephrectomy is the only curative treatment (Robson *et al.* 2002), and currently there is no adjuvant therapy in RCC. Possible treatments for mRCC, in addition to cytoreductive nephrectomy (Flanigan *et al.* 2001, Mickisch *et al.* 2001), are immunomodulators, such as interferon-α (IFN-α) (Kankuri *et al.* 2001, Pyrhönen *et al.* 1999), interleukin-2 (IL-2) (Négrier *et al.* 2007), and more recently tyrosine kinase inhibitors, such as sunitinib (Motzer *et al.* 2007), sorafenib (Escudier *et al.* 2007), and mTOR inhibitor temsirolimus (Hudes *et al.* 2007). Everolimus, another mTOR inhibitor, has an encouraging antitumor activity against mRCC (Motzer *et al.* 2008). The efficacy of bevacizumab, an antiangiogenesis monoclonal antibody, has also been shown when used with IFN-α (Bracarda *et al.* 2011, Rini *et al.* 2010, Yang *et al.* 2003). The Food and Drug Administration (FDA) and EU have also approved pazopanib, an angiogenesis inhibitor, with advanced RCC due to the efficacy of it in RCC (Sternberg et al. 2010). Ongoing clinical trials are addressing the role of targeted agents in adjuvant therapy in RCC (Choueiri *et al.* 2011). The efficacy of many potent novel targeted agents in RCC is under investigation in phase II and III trials, among these axitinib, a multitargeted tyrosine kinase receptor inhibitor (Goldstein *et al.* 2010), tivozanib, a pan-VEGFR tyrosine kinase inhibitor (De Luca and Normanno 2010), and ipilimumab, an anti-CTLA4 antibody (Yang *et al.* 2007). Additionally, vaccine therapy in RCC is being studied (Rini *et al.* 2011). The stabilization of the disease has been shown to be beneficial for the survival of mRCC patients (Thiam *et al.* 2010, Kankuri *et al.* 2001*).*

2. Staging and prognostic factors in RCC

2.1 Pathological tumor staging

In the 1960's, Robson *et al*, created the staging system based on physical characteristics and tumor spread with the addition of tumor venous invasion (Robson *et al.* 2002). The poor correlation between the different Robson stages and survival led to the recommendation to use the TNM (tumor, node, metastases) staging system. Since 1978, the TNM classification system for the extent of the tumor spread has integrated characteristics such as tumor size, vascular involvement, nodal spread and distant metastases (Bassil *et al.* 1985, Harmen 1978). pTNM classification system was updated by the Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC) in 1997 when the cut-off between T1 and T2 tumors was increased from 2.5 cm to 7 cm, in order to increase the difference in survival from these two tumor types. Analysis of outcome in nephrectomized patients showed that the 1997 TNM-system cut-off point between T1 and T2 tumors is too high, and a cut-off point of 4.5 – 5.0 cm has been suggested (Elmore *et al.* 2003, Zisman *et al.* 2001). In 2002, the pTNM classification system was revised: T1 was divided into T1a and T1b by a cut-off point of 4 cm, according to the suitability for partial nephrectomy, and prognostication (Sobin and Wittekind 2002, Guinan *et al.* 1997). A uniform staging classification, the TNM staging system, has improved the division of patients into radical or partial nephrectomy candidates. Additionally, it has increased the co-operation between oncologists and pathologists concerning the outcome of RCC patients (Janzen *et al.* 2003, Javidan *et al.* 1999). Howerer, modifications in the TNM system may cause difficulty in comparing outcome data in different studies (Belldegrun *et al.* 1999, Störkel *et al.* 1989).

Pathological tumor stage (T-stage) has been observed to be the most important factor for locally confined RCC in predicting the survival of patients who have undergone nephrectomy (Kankuri *et al.* 2006, Delahunt *et al.* 2002). The observed five-year survival is approximately 75-80% for stage T1, 55% for T2, 40% for T3, and 20-30% for T4 (Sunela *et al.* 2009, Tsui *et al.* 2000). For patients with stage I disease (tumor confined to the kidney) the five-year survival is approximately 90%, and for those with stage I and histologic of chromophobe type it is almost 100% (Zisman *et al.* 2001). The five-year survival rate for stage III disease is approximately 50% (Zisman *et al.* 2001). There is an 80% difference in survival rates between patients with local disease compared to those with advanced disease and distant metastases (American Cancer Society 2004). In a retrospective review of 2 473 RCC patients from 1975 - 1985, regardless of T-stage, tumor size was observed to have an inverse association with survival (Guinan *et al.* 1995). In the study of Kankuri *et al.* (2006), in the analysis of those RCC patients who later developed metastatic disease, high T-stage caused twice the risk of metastatic disease and three times the risk of death compared with low T-stage which indicates that as the tumor size increases, the more aggressive its growth becomes and the more probable is tumor cell dissemination. T-stage is a prognostic factor for both metastases-free and overall survival in RCC patients.

T-stage can be used in estimating the correct duration and frequency of surveillance of RCC patients after nephrectomy. RCC with a diameter of less than 3.0 cm grows slowly; only 2.5% have metastases during the first three years (Bosniak *et al.* 1995). Therefore, in the treatment of those in whom surgery is contraindicated, careful monitoring (watchful waiting) by computed tomography (CT scan) may be used (Roberts *et al.* 2005, Bosniak *et al.* 1995). Previously, it has been suggested that T-stage is not an important prognostic factor in the survival of patients who have neither lymph node nor distant metastases (Giuliani *et al.* 1990). The therapeutic value of lymph node dissection remains unproven (Mickish 1999). T-stage alone has been pointed to be a valuable prognostic factor for survival, even when the status of lymph nodes is unknown (Kankuri *et al.* 2006). Additionally, a high T-stage has been used as an inclusion criterion for adjuvant treatments in trials (Atzpodien *et al.* 2005, Repmann *et al.* 2003).

Moreover, T-stage is an independent prognostic factor in mRCC patients (Kankuri-Tammilehto *et al.* 2010). In the study of Kankuri-Tammilehto *et al.* (2010) high T-stage caused twice the risk of death compared with low T-stage in mRCC. The association between T-stage and overall survival was also found in those with primary metastases at the time of nephrectomy (Kankuri *et al.* 2006). T-stage is not typically used in prognostic models in mRCC, a UCLA model (Zisman *et al.* 2002) being an exception. T-stage seems to be a good tool in prognostic evaluation in mRCC patients and could be included in prognostic models.

2.2 Histopathological tumor grading

In grading systems, the major criteria are nuclear and nucleolar appearances, while in some systems, tumor architecture and cell type is also included (Mostofi *et al.* 1998, Goldstein 1997, Fuhrman *et al.* 1982, Syrjänen and Hjelt 1978, Skinner *et al.* 1971). The WHO grading system is based on the size and prominence of nucleoli (Eble *et al.* 2004, Mostofi *et al.* 1998), while the Fuhrman grading system is based on nuclear size, shape, and presence or absence of nucleoli (Fuhrman *et al.* 1982). The WHO grading system contains three grades, whereas the Fuhrman contains four.

Several studies have failed to demonstrate any statistically significant differences in the survival of patients with different grades, when all three or four grades are analyzed separately (Kankuri *et al.* 2006, Rioux-Leclercq *et al.* 2000, Usubutyn *et al.* 1998, Selli *et al.* 1983) although when analyzing only the highest and the lowest grades the statistically significant difference in survival have been found (Kankuri *et al.* 2006). This is partly because, as yet, no consensus has been reached on a universal tumor grading system (Kanamaru *et al.* 2001, Medeiros *et al.* 1997). The observed five-year disease-specific survival (DSS) rate is approximately 90% for G1, 70-85% for G2, 45-60% for G3, and 15-30% for G4 (Gudbjartsson *et al.* 2005, Ficarra *et al.* 2001). Currently, different grading systems are utilized at different institutions. Tumor-grading systems have been criticized because of their subjectivity in tumor evaluations (Lanigan *et al.* 1994), and comparison of different patient cases with respect to histopathological grade is difficult. More quantitative measures which describe the size or the shape of the nuclei have been requested by pathologists. In 1997, an international consensus conference on RCC by UICC and AJCC outlined recommendations for the grading of RCC (Goldstein 1997): the grading system should be based on standardized and reproducible criteria that reflect the heterogeneity of nuclear and nucleolar features within a tumor, and each grade should result in significant differences in patient outcome. Recently again, a joint group of urologists and pathologists has published a proposal that the criteria for nuclear grading should be different for the different histopathologic subtypes of RCC according to the Heidelberg classification (Paner *et al.* 2006). Additionally, reducing the grades in the Fuhrman system has been proposed, for better outcome stratification (Rioux-Leclercq *et al.* 2007, Lohse *et al.* 2002, Bretheau *et al.* 1995). Overall, histopathological grade seem to be imprecise for prognostic evaluation in RCC patients (Uchida *et al.* 2002, Rioux-Leclercq *et al.* 2000, Lanigan *et al.* 1994).

2.3 Heidelberg and WHO classifications for typing of renal tumors

In Heidelberg, in October 1996, the morphology was combined with genetic findings for a new classification, called the Heidelberg classification of renal tumors, in a workshop organized by the UICC and the AJCC (Kovacs *et al.* 1997, Störkel *et al.* 1997). In addition to this, in 2004, WHO published the reassessed classification which is now based on both genetic and pathological abnormalities (Eble *et al.* 2004). Progress in our knowledge of genetic alterations leads to new suggestions for RCC entities (Eble 2003). With the progress of research, the Heidelberg classification may lead to more specific treatments in different subgroups of RCC patients. The 5-year DSS for locally confined RCC is for chromophobe RCC approximately 87-100%, for papillary RCC 87%, and for conventional RCC 70-75% (Cheville *et al.* 2003, Amin *et al.* 2002). In the case of sarcomatoid change, the survival decreases with the 5-year DSS of 35% (Amin *et al.* 2002). A very rare entity of collecting duct RCC is highly aggressive with highly decreased prognosis (Antonelli *et al.* 2003). The prognostic power of the Heidelberg classification has been investigated. The current Heidelberg classification does not have independent prognostic ability, and thus it should not be considered as a major prognostic variable comparable to T-stage and histopathological tumor grade (Patard *et al.* 2005). However, Heidelberg classification associates with metastases development, indicating that unclassified tumor type metastasizes with high probability (Kankuri *et al.* 2006). In future, with the progress of research, the Heidelberg classification may lead to more specific treatments in different subgroups of RCC patients (Störkel *et al.* 1997).

2.4 Prognostic models in RCC

The heterogeneity of RCC within the same T-stage and grade (Tsui *et al.* 2000) has resulted in a need for prognostic models for prognostication and treatment modality selection. Prognostic models, anagrams and nomograms, have been developed to find those nephrectomized RCC patients who potentially have a long-term recurrence-free interval and survival, as well as those mRCC patients who have long-term survival (Table 1.). The most often represented as an independent prognostic factors in metastatic RCC (mRCC) are performance status, time to metastases, number of metastatic sites, and prior nephrectomy. Therapies for mRCC cause a wide variety of adverse effects, which reduce the quality of life. Determining the prognostic factors for survival in mRCC patients is valuable in directing therapy for those patients who would benefit from it. Several models have been developed for predicting the likelihood of response to therapy and to predict survival. However, novel biomarkers are hoped to specify the diagnosis, staging, and prognosis and to guide targeted cancer therapies. Molecular tumor markers are expected to revolutionize the staging of RCC in the future (Srigley *et al.* 1997), as nowadays stratifying the patients into risk groups is largely done on the basis of clinopathological factors, e.g. clinical stage of the disease. Still, all the molecular mechanisms that affect the development, progression and clinical behavior of RCC are not known. Advances in the understanding of the pathogenesis, behavior, and molecular biology of RCC may help to better predict tumor prognosis, and thus improve survival of RCC carcinoma patients when a more tailored therapy can be given to each individual patient. Molecular biomarkers, such as p53, Ki-67 and COX-2, are candidates for defining prognostic subgroups (Delahunt *et al.* 2002), and for guiding targeted therapies (Masters 2007), as shown in the studies, where p53, Ki-67 and COX-2 had prognostic value in predicting survival. The following chapters describe in more detail about the value of them in the prognosis in RCC.

3. Biomarkers related to molecular mechanism in RCC

3.1 pVHL, von Hippel-Lindau protein, mudulator of hypoxic response

pVHL, a tumor suppressor gene product, is expressed especially in the kidney's proximal renal tubule (Corless *et al.* 1997, Iliopoulos *et al.* 1995). Approximately 61-75% of sporadic conventional RCCs contain mutations in *VHL*, in the short arm of chromosome 3 (3p25-26) (van Houwelingen *et al.* 2005, Maxwell *et al.* 1999, Gnarra *et al.* 1994,), of which 50% show loss of heterozygosity (LOH) (Kovacs *et al.* 1997, Gnarra *et al.* 1994) and 10-20% silencing of the wild-type allele by promoter hypermethylation (Herman *et al.* 1994). VHL is associated with carcinogenesis. The function of pVHL is ubiquitylation of hypoxia-inducible factor (HIF); therefore, it modulates the hypoxic response; VHL protein can bind to hypoxia inducible factor-1 alpha (HIF-1α) and target this factor for destruction in the presence of oxygen. HIF in turn controls the expression of several proteins, including carbonic anhydrase 9 (CA9) and proteins involved in angiogenesis, i.e. vascular endothelial growth factor (VEGF) and EPO, via oxygen-dependent ubiquitination (van Houwelingen *et al.* 2005, George and Kaelin 2003). Normally, VHL down regulates vascular endothelial growth factor (VEGF) by different pathways. In VHL-defective cancer cells, increased concentrations of VEGF and EPO are observed.

Reference	Year	No. of Patients	Therapy Administered	Tumor Subtype	Prognostic Factors	Prognostic Information
Motzer *et al.* (MSKCC)	2002	463	IFN-α	All	Performance status, time from diagnosis to start of therapy, LDH, hemoglobin, corrected calcium	Survival
Zisman *et al.* (UCLA)	2002	262	IL-2 or IFN-α (197 pts), other (65 pts)	All	T-stage, nodal involvement, nuclear grade, no. of symptoms, immunotherapy	Survival
Négrier *et al.* (Group Francais d'Immunotherapie)	2002	782	IFN-α ± IL-2	All	Performance status, no. of metastatic sites, disease-free interval, signs of inflammation, hemoglobin	Survival, rapid progression
Atzpodien (Medizinische Hochschule Hannover)	2003	425	IFN-α + IL-2 ± 5-FU ± 13CRA	All	Neutrophil count , LDH, CRP, time from diagnosis to start of therapy, no. of metastatic sites, bone metastases	Survival
Motzer *et al.* (MSKCC)	2004	251	New agents	All, if cytokine refractory disease	Performance status, hemoglobin, corrected calcium	Survival for those who enter clinical trials of new agents
Choueiri *et al.* (Cleveland Clinic Foundation)	2007	358	IFN-α ± IL-2 ± chemotherapy	All	Performance status, hemoglobin, no. of metastatic sites, involved kidney of primary tumor	Long-term survival
Cho et al (Yonsei University)	2008	197	Immunotherapy	All	Performance status, N stage, no. of metastatic sites, sarcomatoid differentiation, liver metastasis	Survival
Motzer *et al.* (MSKCC)	2008	375	Sunitinib	Conventional RCC	Performance status, time from diagnosis to start of therapy, nephrectomy status, no. of metastatic sites, presence of liver or lung metastases, LDH, corrected calcium, hemoglobin, alkaline phosphatase, thrombosytosis	Probability of 12-month progression-free survival

LDH=lactate dehydrogenase
MSKCC = Memorial Sloan Kettering Cancer Center
UCLA = University of California

Table 1. Prognostic algorithms and nomograms for survival in mRCC between 2000 and 2008.

3.2 CA9, hypoxia associated enzyme

CA9, a member of the carbonic anhydrase family, is suggested to play a role in the regulation of cell proliferation in response to hypoxic conditions. Low CA9 expression associates with the absence of VHL mutation and aggressive tumor characteristics in

conventional RCC (Pantuck *et al.* 2007). CA9 may indicate those patients who benefit from IL-2, as low CA9 expression associates with lower survival compared to high CA9 expression in mRCC patients who receive IL-2 (Atkins *et al.* 2005, Bui *et al.* 2003). It has also been suggested that CA9 may indicate those patients who benefit from CA9-targeted therapies. It is also being investigated whether CA9 may indicate those patients who are potential candidates for adjuvant therapy.

3.3 p53, biomarker of cell cycle point

p53, a tumor suppressor gene product, is a promoter of cell growth arrest and apoptosis (Choisy-Rossi and Yonish-Rouach 1998). Activated p53 elicits several cellular responses, including apoptosis and cell cycle arrest (Reich and Levine 1984), and responds to DNA damage at the restriction checkpoint of the G1 phase of the cell cycle (May and May 1999). In normal cells, p53 is usually undetectable (Finlay *et al.* 1988). Mutant p53 accumulates in cell nuclei and can be immunostained (Reich and Levine 1984), whereas wild-type p53, because of its short half-life, is usually undetectable by routine immunohistochemistry (Reich and Levine 1984). p53 accumulation and increased cell proliferative activity are parallel phenomena in RCC (Kankuri *et al.* 2006, Pinto *et al.* 2005). p53 may be upregulated in part by VHL, accounting for some of the tumor suppressive functions of VHL in RCC (Galban *et al.* 2003). p53 seems to associate weakly with tumor grade, as the association was seen only in univariate analysis. Nor was an association between p53 and grade observed in a previous microarray study (Zigeuner *et al.* 2004). In both studies, the nuclear grade was determined according to the WHO guidelines.

Published results on the association of p53 with survival have been controversial, some studies suggesting positive p53 associating with poor survival (Shvarts *et al.* 2005 , Zigeuner *et al.* 2004, Uchida *et al.* 2002, Haitel *et al.* 2000), while others have observed no association (Itoi *et al.* 2004, Olumi *et al.* 2001, Rioux-Leclercq *et al.* 2000, Hofmockel *et al.* 1996). In the study of Phuoc *et al.* (2007), p53 was significantly associated with survival in univariate analysis, but the association was not independent. In a tissue array study on metastasized patients, overexpression of p53 was associated with impaired DSS in renal carcinoma (Kim *et al.* 2004). In some studies, the association of p53 and survival has been investigated in a group of RCC patients with both locally confined and primary metastatic RCC; thus, patient selection varies in different studies (Olumi *et al.* 2001). The study of Kankuri *et al.* (2006) indicates that p53 is not able to predict which patients will develop metastatic disease after nephrectomy, but interestingly, they predict poor survival in mRCC patients (Figure 2.). Therefore, p53 can help in determining metastatic patients with a poor prognosis and, e.g. those who might benefit from aggressive treatment, such as high-dose interleukin-2 (Spanknebel *et al.* 2005) or temsirolimus (Hudes *et al.* 2007).

3.4 Ki-67, proliferation marker

Ki-67, a proliferation biomarker, is expressed throughout the active phases of the cell cycle, and serves as a good marker for proliferative activity in cell nuclei (Gerdes *et al.* 1984). Ki-67 accumulates during the cell cycle from G1 to mitosis, and is at its lowest level after mitosis (du Manoir *et al.* 1991). The percentage of nuclei staining by immunohistochemistry reflects Ki-67 expression (Olumi *et al.* 2001). An association between Ki-67 and high T-stage and metastases development have been observed

(Kankuri *et al.* 2006, Dudderidge *et al.* 2005, Rioux-Leclercq *et al.* 2000), indicating that Ki-67 is a marker for aggressive disease in RCC with an increased risk of early metastases development. Ki-67 has been reported to independently predict survival following nephrectomy in many studies (Dudderidge *et al.* 2005, Bui *et al.* 2004, Itoi *et al.* 2004, Rioux-Leclercq *et al.* 2000, Aaltomaa *et al.* 1997). Ki-67 has been observed to increase in sarcomatoid change (Kanamaru *et al.* 1999), indicating different protein expression profiles in different entities according to the Heidelberg classification.

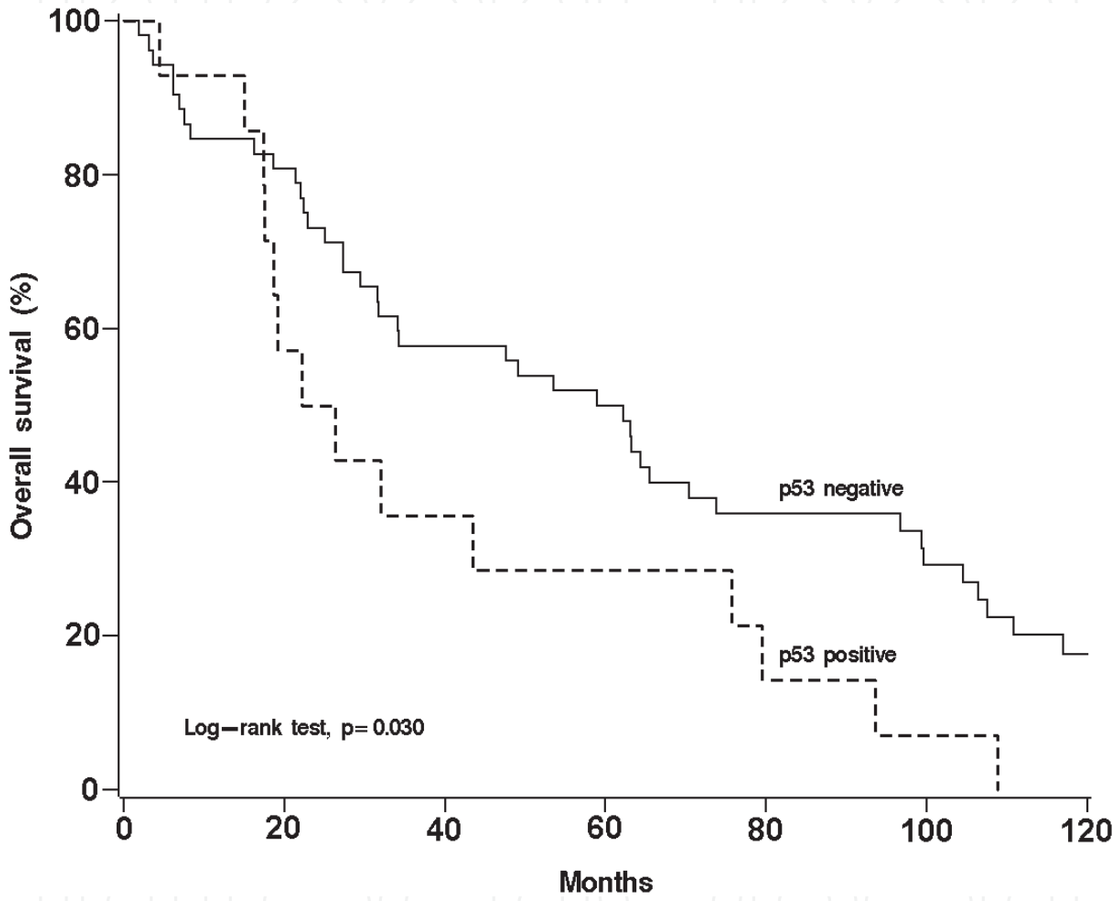


Fig. 2. Kaplan-Meier survival curve for p53 in mRCC (n=66) (Kankuri *et al.* 2006).

Dudderidge *et al.* (2005) found Ki-67 to be an independent prognostic factor for disease-free survival in nephrectomized RCC, but opposite results have also been published (Donskov *et al.* 2004, Kim *et al.* 2004, Yildiz *et al.* 2004). No association between Ki-67 alone and survival in locally confined RCC patients was found in the study of Kankuri *et al.* (2006). The differences in the classification of metastases are seen: Kim and coworkers (2004) classified both distant and local lymph node metastases as metastatic disease, whereas in the study of Kankuri *et al.* (2006), only tumors with distant metastases were classified as metastatic. However, Ki-67 predicts poor survival in mRCC patients (Figure 3.). Therefore, in addition to p53, Ki-67 can help in determining metastatic patients with a poor prognosis and, e.g. those who might benefit from aggressive treatment.

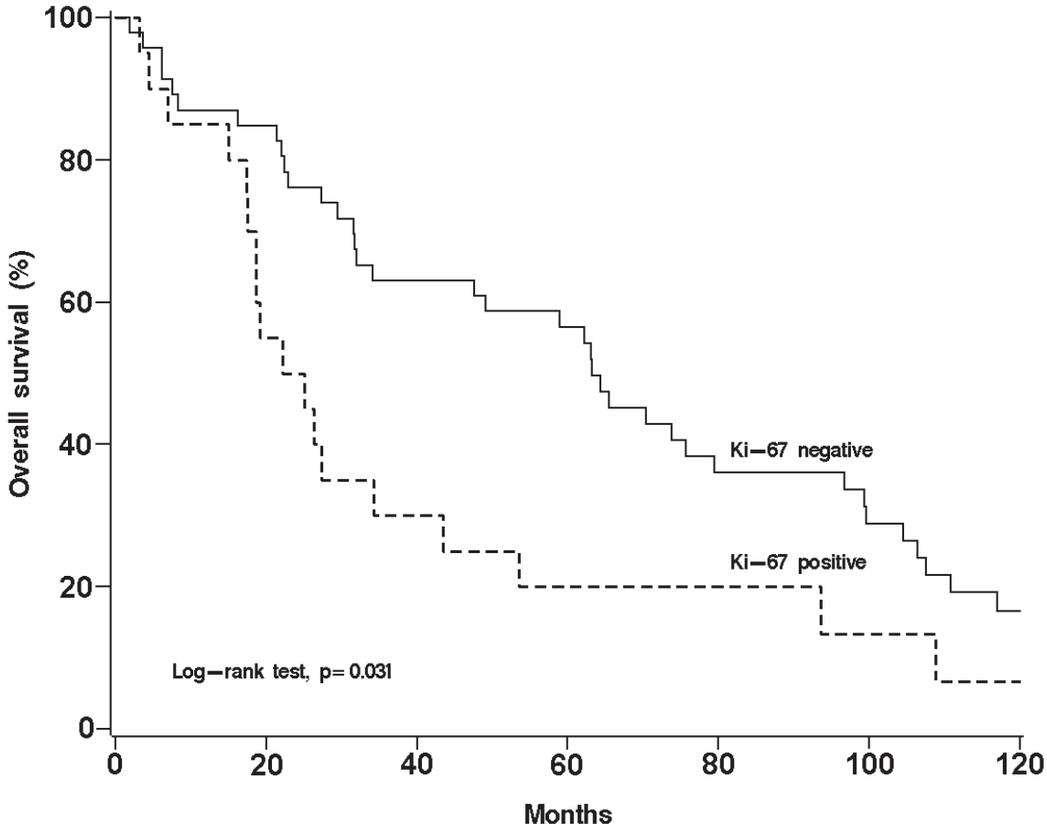


Fig. 3. Kaplan-Meier survival curve for Ki-67 in mRCC (n=66) (Kankuri *et al.* 2006).

3.5 COX-2, biomarker for inflammation and neoplasia

Cyclo-oxygenase-2 (COX-2), an isoform of the COX[3] enzyme, is an inducible form of an enzyme involved in the first steps of prostaglandins and thromboxane synthesis. COX-2 converts arachidonic acid first into prostaglandin G2, and afterwards by peroxidase activity into prostaglandin H_2, a precursor of the prostaglandins (Taketo 1998). COX-2 is suggested to play a physiological role in fetal nephrogenesis (Khan *et al.* 2001). COX-2 increases in inflammation and neoplasia (Miyata *et al.* 2003, Hara *et al.* 2002, Nose *et al.* 2002, *et al.* Taketo 1998), and is undetectable in most normal tissues (Mungan *et al.* 2006, Yoshimura *et al.* 2004). The conversion of procarcinogens to proximate carcinogens is catalyzed by the peroxidase activity of COX-2 (Elinq *et al.* 1990). COX-2 is highly induced by stimulus of oncogenes, cytokines, growth factors, and tumor promoters (Smith *et al.* 2000, Herschman 1996, Subbaramaiah *et al.* 1996). Associations between COX-2 over-expression and antiapoptotic ability, tumor invasiveness, tumor growth, angiogenesis, and immunosuppression, as well as multidrug resistance in cancer have been reported (Cao and Prescott 2002, Masferrer *et al.* 2000, Subbramaiah *et al.* 1996, Tsujii and DuBois 1995).

Cytoplasmic/membranous COX-2 staining by immunohistochemistry reflects COX-2 protein expression (Cho *et al.* 2005). The study results on associations of COX-2 with tumor stage, grade, and survival have been contradictory. Yoshimura *et al.* (2004) demonstrated that COX-2 was expressed at its highest in G1, as well as in pT1 RCC tumors, compared to other RCC

tumors in grade and T-stage, while in Hashimoto *et al*'s study (2004), more COX-2 was found at the higher tumor grade, as well as stage. Kankuri-Tammilehto *et al.* (2010) found no association between COX-2 and tumor grade or T-stage. A significant association has been observed between COX-2 and Ki-67 expression in the study of Miyata *et al.* (2003), whereas Kankuri-Tammilehto *et al.* (2010) found no association between them. No association between COX-2 and p53 has been found in studies (Kankuri-Tammilehto *et al.* 2010, Cho *et al.* 2005).

Kankuri-Tammilehto *et al.* (2010) found that the proportion of COX-2 positive tumors is highest in RCC with the ability to develop later metastases, when compared to both RCC without metastatic potential and RCC with primary metastases. This finding was new. Previously, Miyata *et al.* (2003) observed that positive COX-2 expression associated with primary metastases in univariate analysis (when M0-patients were compared to M1-patients). Cho *et al.* (2005) found no association between positive COX-2 expression and metastases (when M0-patients were compared to M1-patients, or appearance of metastatic disease was compared to non-metastatic disease). In those studies, the method of analysis differs from that of the study of Kankuri-Tammilehto *et al.* (2010), where patients were divided into three categories according to the appearance of metastases. According to the study of Kankuri-Tammilehto *et al.* (2010), metastases-free survival is longer in patients with COX-2 positive tumors. The median metastases-free survival was 46 months in RCC with COX-2 positivity compared to 15 months in RCC with COX-2 negativity (Figure 4.). These

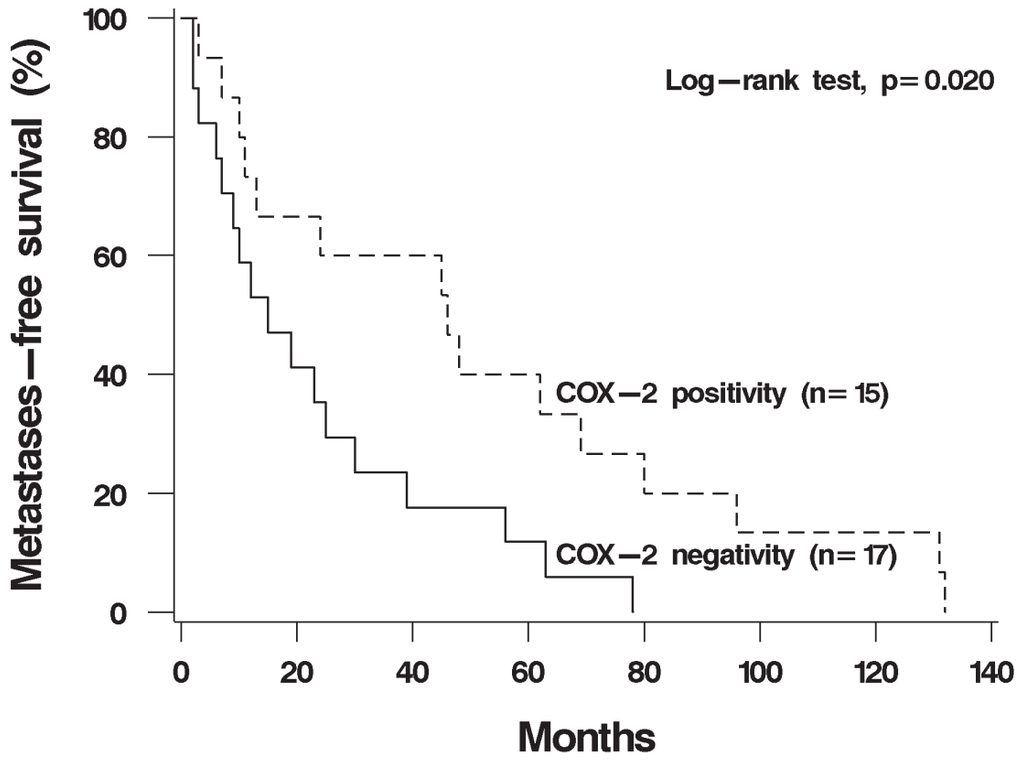


Fig. 4. The prognostic value of COX-2 for metastases-free survival from nephrectomy in RCC patients who later developed metastatic disease (n=32, Kaplan-Meier method): the median metastases-free survival time was 46 months with COX-2 positivity, and 15 months with COX-2 negativity (Kankuri-Tammilehto *et al.* 2010).

results indicate that COX-2 positivity associates with the delay of metastatic formation in RCC patients who do not have disseminated disease at presentation, and that COX-2 negativity associates with an aggressive phenotype in mRCC disease.

Few studies have reported the results of an association between COX-2 expression and survival in RCC patients. Previously, Miyata *et al.* (2003) found that the five-year survival of patients with COX-2 positive tumors from nephrectomy was 66%, and of COX-2 negative patients 91% (Miyata *et al.* 2003). In Miyata's study, the patients were 86% M0 and 14% M1 at nephrectomy. Previously, no results of COX-2 and overall survival in mRCC patients have been published. The study of Kankuri-Tammilehto *et al.* (2010) indicates that COX-2 positivity predicts improved overall survival in patients with mRCC treated with IFN-α. This is in line with the previous study of Rini *et al.* (2006), in which COX-2 positivity associated with longer time to progression in the patients treated with celecoxib plus interferon-α. Kankuri-Tammilehto *et al.* (2010) observed no association between COX-2 staining and response to IFN-α alone, while Rini *et al.* (2006) reported that all the RCC patients with objective responses to celecoxib plus interferon-α expressed COX-2 staining. Additionally, COX-2 does not associate with the Heidelberg classification (Kankuri-Tammilehto *et al.* 2010, Yoshimura *et al.* 2004).

3.6 Her-2, biomarker of proto-oncogene product

Her-2, a proto-oncogene product, is a member of the ErbB family of receptor tyrosine kinases. Her-2 functions in secretory epithelial tissues, and regulates intracellular signaling cascades (Arteaga *et al.* 2001, Olayioye *et al.* 2000). Her-2 is over-expressed in approximately 20-30% of human adenocarcinomas (Latif *et al.* 2002, Lipponen *et al.* 1994, Slamon *et al.* 1989), and the over-expression is associated with metastatic phenotype and poorer prognosis, e.g. in breast and ovarian cancer (Slamon *et al.* 1989).

Gene amplification of *Her-2* can be investigated by cytogenetic analyses, such as fluorescent *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and polymerase chain reaction (PCR). In breast cancer, FISH and CISH positivity are accurate predictors of response to trastuzumab (anti-Her2 therapy) (Isola *et al.* 2004, Lebeau *et al.* 2001). Receptor-mediated targeted tumor therapy with Herceptin® (RhuMAb HER-2), a recombinant humanized monoclonal anti-Her-2 antibody, has improved the survival of breast carcinoma patients both in adjuvant therapy and in therapy for metastatic disease (Smith *et al.* 2007, Montemurro *et al.* 2003).

Membranous staining of HER-2 in immunohistochemistry reflects HER-2 protein expression (Zhang *et al.* 1997). Her-2 receptor-specific tumor toxin, in an animal model, effectively reduced pulmonary tumors of advanced RCC (Maurer-Gebhard *et al.* 1998). Parallel associations of Her-2 expression between tumor stage and grade in RCC patients have been observed in many studies (Zhang *et al.* 1997, Stumm *et al.* 1996), although in the study of Seliger *et al.* (2000) no such association was found. In the study of Hofmockel *et al.* (1997), higher tumor grades were seen when Her-2 expression was low, and higher T-stage associated with high Her-2. In the study of Phuoc *et al.* (2007), Her-2 protein expression did not correlate with Ki-67 protein expression.

In most *HER-2* gene amplification studies, *Her-2* gene amplification was observed neither by FISH analysis (Latif *et al.* 2002), messenger ribonucleic acid (mRNA) analysis (Stumm *et al.*

1996), nor PCR analysis (Selli *et al.* 1997, Zhang *et al.* 1997). Selli *et al.* (1997) found *HER-2* gene amplification in collecting duct RCC cases (45%). Therefore, *HER-2* gene amplification may be more pronounced in collecting duct RCC, than in other more common RCC types (Matei *et al.* 2005, Zhang *et al.* 1997). The association of *HER*-2 gene amplification and HER-2 protein expression with the prognosis of RCC patients has been estimated in few studies and the results have been contradictory (Phuoc *et al.* 2007, Lipponen *et al.* 1994). Further studies are needed to determine whether HER-2 protein expression or *HER-2* gene amplification may be used as prognostic factors in RCC patients.

3.7 Incidence of p53, Ki-67, and COX-2 expressions

The incidence of p53- and Ki-67-positive expression in RCC tumors was low in RCC studies (Kankuri-Tammilehto *et al.* 2010, Kirkali *et al.* 2001, Haitel *et al.* 2000, Rioux-Leclercq *et al.* 2000). It is known that in addition to melanoma, RCC belongs to tumors with a low incidence of *p53* mutations compared to, e.g. prostate and bladder cancer (Haitel *et al.* 2000, Kirkali *et al.* 2001, Rioux-Leclercq *et al.* 2000). The low *p53* mutation in different cancers (Olivier *et al.* 2002) and the low immunohistochemical staining of RCC tissue blocks for the p53 protein in studies (Haitel *et al.* 2000, Rioux-Leclercq *et al.* 2000) suggest that mutations in *p53* result in an accumulation of the p53 protein. In the study of Oda *et al.* (1995), p53 expression was found only in those components with *p53* mutations, mainly in the sarcomatoid components. The 10% cut-off value of p53 and Ki-67 was often selected to achieve statistically reliable results, and in accordance with previous studies on the subject (Kankuri *et al.* 2006, Olumi *et al.* 2001). Previously published reports indicate that the proportion of COX-2 positive cells varies in human RCCs (Cho *et al.* 2005, Miyata *et al.* 2003). In the study of Kankuri-Tammilehto *et al.* (2010), weak intensity of COX-2 staining was considered as COX-2 negative, which resulted in a lower number of positive COX-2 cells than in some other RCC studies (Tuna *et al.* 2004, Cho *et al.* 2005). For comparison, in the study of Miyata *et al.* (2003), the criterion for positive COX-2 expression was 5%, whereas in the study of Kankuri-Tammilehto *et al.* (2010), it was considered to be 10%. Also different antibodies have been used in other studies (Rini *et al.* 2006, Cho *et al.* 2005, Hashimoto *et al.* 2004). This fact and the criteria for immunohistochemical classification may contribute to the difference in the results. Validation of immunohistochemical methods is needed before the methods could be widely adopted for in clinical use.

3.8 Combining markers

In multivariate analysis, COX-2 and Ki-67 were independent variables, indicating that they are both stronger biomarkers than p53 for the development of metastases in RCC. However, combining markers may specify prognostic subgroups better than observing a single marker. As shown in a study by Haitel *et al.* (2000), p53 was not an independent predictor for survival, but p53 and mdm2, a negative regulator of p53, showed a strong association with poor survival. In the study of Kankuri *et al.* (2006), in RCC patients, double positivity for p53 and Ki-67 expression seems to indicate a higher probability of metastases than either marker alone. Additionally, combining COX-2 and Ki-67 increases their ability to predict survival in mRCC (Figure 5.). In this study, median overall survival time of RCC with COX-2 negativity/Ki-67 positivity was 19 months, which was almost five times shorter than of RCC with COX-2 positivity/Ki-67 negativity. Median overall survival time of RCC with

COX-2 negativity alone was 28 months, which was three times shorter than that of RCC with COX-2 positivity.

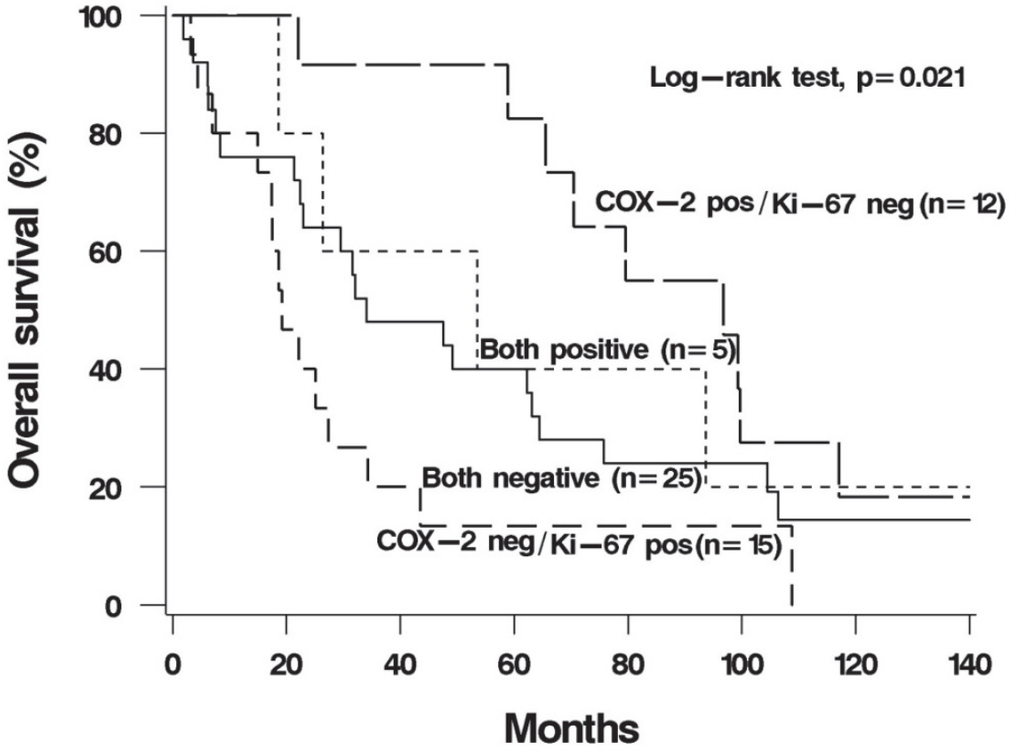


Fig. 5. The prognostic value of covariation of COX-2/Ki-67 for overall survival from nephrectomy in RCC patients with metastases (either primary presentation or later) (n=57, Kaplan-Meier method): the median overall survival time was 97 months with COX-2 positivity/Ki-67 negativity, and 19 months with COX-2 negativity/Ki-67 positivity (p=0.004) (Kankuri-Tammilehto *et al.* 2010).

Prognostic markers can be used in patient counseling, to select treatment modalities, and to determine eligibility for clinical trials. Different prognostic models have been created to specify the prognosis of RCC patients; they typically include conventional prognostic markers. However, combining biomarkers and conventional clinical markers seems to predict DSS more accurately than grade or TNM stage alone, both in locally confined and metastatic RCC (Kim *et al.* 2004).

3.9 Trends in the use of biomarkers

Prospective clinical trials on the clinical use of p53, Ki-67, and COX-2 protein expression in predicting overall survival could answer the question of whether the expression of these biomarkers can be reliably used in mRCC. These biomarkers cannot predict response to IFN-α (Kankuri-Tammilehto *et al.* 2010). Whether these biomarkers can predict response to novel targeted therapies should be investigated in trials. The new era of genetic cancer studies shows great promise in terms of patient evaluation for new targeted therapies or immunotherapy. By means of the tissue microarray technique, thousands of tumors can be

investigated simultaneously to determine the protein expression profile. However, creating a consensus in the tissue microarray construction protocol is challenging, as RCC is a relatively large-size tumor of a highly heterogenous nature (Signoretti *et al.* 2008). At current, whole tissue sections are considered the gold standard, but the more cores per tumor are sampled the fewer errors are introduced by limited sampling. Using gene chips to profile kidney tumors defines the genes that determine patient survival and response to therapy, thus enabling precise prognosis determination and individual treatment planning (Tan *et al.* 2008). Additionally, tissue microarrays enable the analysis of protein expression profiles in specimens to determine their potential clinical significance and role in RCC biology.

4. Conclusion

RCC is an extremely heterogeneous disease, with patients having an overall survival from a few months to several years. For those RCC patients with performance status enabling current treatments, such as nephrectomy, immunomodulators, and more recently targeted therapies, the expected five-year survival rate has been slightly higher than 60%. Metastatic disease is seen in 20-30% of RCC patients at diagnosis. The five-year survival for metastatic RCC is from 3% to 16% if metastasectomy has not been a possible treatment. Currently, tumour (T)-stage is the best known prognostic factor for locally confined RCC. T-stage is a prognostic factor for both metastases-free and overall survival in locally confined RCC patients as well as in overall survival in metastatic RCC (mRCC). No consensus has been reached on a universal histopathologic tumor grading system. Several published reports have pointed out the differences in survival between the highest and the lowest tumor grades, even though when all three or four tumor grades were analyzed separatedly, the differences were no longer statistically significant. The heterogeneity of RCC within the same T-stage and grade has resulted in a need for more specific prognostic markers, related to molecular mechanisms of RCC, to specify diagnosis, staging and prognosis. Prognostic markers can also be used in to select treatment modalities, help in surveillance, and to determine eligibility for clinical trials. p53 associates weakly with tumor grade whereas Ki-67 associates with T-stage and metastatic development, indicating that Ki-67 is a marker for aggressive disease in RCC with an increased risk of early metastases development. The proportion of COX-2 positive tumors is highest in RCC with the ability to develop later metastases, when compared to both RCC without metastatic potential, and RCC with primary metastases. Metastases-free survival is longer in patients with COX-2 positive tumors compared to COX-2 negative tumors. These data show that COX-2 negativity associates with an aggressive phenotype in mRCC disease. COX-2 and Ki-67 alone are stronger biomarkers than p53 for the development of metastases in RCC. Her-2 seems to associate with p53 and Ki-67, but results of associations between Her-2 and survival have been contradictory. Few studies have been published on the significance of Her-2 protein expression or *Her-2* gene amplification in RCC, so more studies are warranted. p53 or Ki-67 alone are not valuable prognostic markers in locally confined RCC, but they can predict poor survival in mRCC. Therefore, p53 and Ki-67 can help in determining metastatic patients with a poor prognosis and, e.g. those who would benefit from high-dose IL-2 or temsirolimus. COX-2 positivity predicts improved overall survival in patients with mRCC treated with IFN-α. p53, Ki-67, and COX-2 cannot predict response to IFN-α. Investigating the ability of p53, Ki-67, and COX-2 protein expression to predict overall survival in a

prospective clinical trial would answer the question of whether these biomarkers can be reliably used in mRCC. Combining the results of COX-2 and Ki-67 expression, may predict overall survival in mRCC. In predicting the development of metastases in nephrectomized RCC patients, COX-2 alone or a covariation of p53 and Ki-67 seem to have prognostic value. Combining p53 or COX-2 with Ki-67 may result in more specific prognosis staging in RCC than observing a single marker. In future, using the tissue microarray technique, the protein expression profile with several biomarkers can be determined quickly. Further investigations are needed on the reproducibility of staining of these novel biomarkers, and on validation of the ability of the expressions to discriminate clinical outcome in RCC. Findings on novel biomarkers have increased our understanding of the molecular biology of locally confined RCC patients and metastatic RCC patients. RCC is characterized by high resistance to radiation and chemotherapy, which may be due to the suppression of apoptotic mechanisms, such as the p53 tumour suppressor pathway. In RCC patients, Ki-67 expression is not very high, which may partly explain RCC's resistance to chemotherapy. Specification of the roles of novel tumor-related molecular prognostic factors might be translated into prognostic tools that could be used in clinical work.

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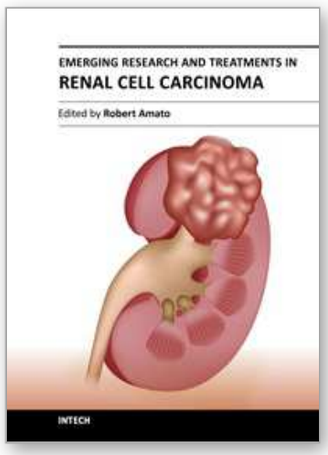

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The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

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