Medizinische Universität Graz

Diploma-Thesis

Accordance between frozen section and paraffin section diagnosis in renal cell carcinoma

a single center analysis of 3140 renal cell carcinoma patients

submitted by

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For the Academic degree of

Doktor der gesamten Heilkunde

(Dr. med. univ.)

at the

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2015

Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of "Good Scientific Practice".

Graz, 03.02.2015

Stefan Hatzl eh

Dedication

This thesis is dedicated to
my grandfather

Karl Hatzl
(*1926 - †2011)

Acknowledgment

At first I would like to thank my supervisors Univ. Prof. Dr. med. univ. Richard Zigeuner and OA PrivDoz. Dr. med. univ. Thomas Chromecki for giving me the opportunity to write this thesis. Special thanks go to OA Priv Doz. Dr. med. univ. Thomas Chromecki, for kindly mentoring, helpful advices and patience not only during this work, also in nearly all the time of my medical education.

Special appreciations go to my family who always supported me in every possible way. Also my friends, here off all Maria Haring, Thomas Rösel, Markus Zoppoth and Andreas Forster were ever faithful and helpful companions through the sometimes hard times during the studies and life.

Abstract

Purpose: One of the basic principles in partial nephrectomy is the resection of the tumor with negative margins verified by frozen section analysis. In cases of positive tumor margins the surgeon is committed to complete the local resection or to perform radical nephrectomy. In this study I retrospectively evaluated the accordance between the yields of frozen section analysis performed during partial nephrectomy and the yields of final pathologic reports, with regard to the compatibility of histological tumor types and positive surgical margins.

Material and Methods: I retrospectively re-evaluated567pathology report of patients undergoing partial nephrectomy with localized renal cell carcinoma from 1994-2013. I stratified the patients into a "frozen section analysis"-group (FSA) and "non - frozen section analysis" –group (non FSA). In 442 patients (78%) frozen section was performed during partial nephrectomy and in125 patients (22%) no frozen section analysis was done. These two groups were compared using χ^2-test, ANOVA, log-rank test and Mann–Whitney U test regarding paraffin embedded pathology, local recurrence and cancer specific survival. I further investigated the histological tumor types, which have been misinterpreted by the pathologist in the "frozen section analysis" group using the χ^2-test and ANOVA.

Results: A total of 567 patients (mean age 60.8 years ± 17.7 years, 66.6% male) underwent intended partial nephrectomy (mean tumor size 3.3cm ± 1.8 cm) from 1994 to 2013.Comparing tumor characteristics between FSA and non-FSA groups, no differences with regard to histological types, tumor grade (Fuhrman), tumor stage, vascular invasion, sarcomatoid differentiation and tumor size were observed between groups.

Frozen section margins were positive in 103 patients (23.3%) in the "FSA"-group. Final margins were positive overall in 54/567 (9.5%), including 7 /125 patients (5.6%) in the "nonFSA"-group compared to 47/442patients (10.6%) in the "FSA"-group ($p = 0.09$).75 patients underwent immediate radical nephrectomy for positive margins in the FSA group. Final pathology revealed residual tumor in 22/75(29%) and no tumor in 53/75 (71%) kidneys. Thus, FSA altered surgical management in 75/442 (17%) operations, in 22/442 (5%) with potential benefit for the patient whereas in 53/442 (12%) cases FSA resulted in overtreatment. Frozen section analysis regarding margin status yielded a sensitivity of 74.5%, a specificity of 82.8%, positive predictive value was 34% and negative predictive value was 96.5%.

Overall, 20/492 (4%) patients developed local recurrence after partial nephrectomy, including 17/367(4.6%) in the “FSA”-group and 3 (2.4%) in the “nonFSA”-group (p=0.33).4patients with a local recurrence had PSM and 13 a negative surgical margin in the final pathology. Cancer specific survival rates were comparable between FSA and non-FSA-groups.

Regarding tumor entities, frozen section and final pathology diagnosis were concordant in 88% (400/442) of the cases in which frozen section was performed. Of the 42 (10%) discordant cases, 4 (10 %) were over diagnosed and 38 (90%) were under diagnosed. Univariable analysis did not reveal any correlation with the accuracy of frozen section diagnosis and patient age, tumor size, presence or absence of sarcomatoid differentiation, the side of tumor localization, grading or staging.

Conclusion: Partial nephrectomy for treatment of RCC can be safely performed with sufficient parenchymal excision without frozen section. Due to its low positive predictive value regarding margin status we cannot recommend routinely frozen section in the treatment of renal lesion, since it does not seem to impact on oncological outcomes and may even result unnecessary nephrectomies.

Zusammenfassung

Zweck: Einer der grundlegenden Prinzipien währendder Nierenteilresektion ist die Entfernung des Nierentumors in sano mit einer Manschette gesunden Nierengewebes, welches den Tumor umgibt. Ob der Nierentumor vollständig oder unvollständig entfernt wurde kann durch eine intraoperative Gefrierschnittanalyse verifiziert werden. Im Falle eines positiven Resektionsrandes ist der Chirurg angehalten das Tumorbeet nachzuresezieren oder die komplette Niere zu entfernen. Jedoch ist es umstritten, ob die Durchführung eines Gefrierschnitts die Rate an positiven Resektionsrändern vermindern kann bzw. die Lokalrezidivrate vermindern kann. In dieser retrospektiven Studie reevaluiere ich die Übereinstimmung zwischen den Ergebnissen der intraoperativen Gefrierschnittanalyse und der endgültigen pathologischen Befunde. Zusätzlich untersuche ich ob die Gefrierschnittanalyse mit der endgültigen Histologie bezüglich der histologischen Tumortypen und der positiven Resektionsränder übereinstimmt.
Material und Methoden: Wir haben 567 Patienten, die zwischen 1994 und 2013 mittels Nierenteilresektion wegen eines lokalisierten Nierenzellkarzinoms operiert worden sind, reevaluiert.
Jene Patienten die einen intraoperative Gefrierschnittanalyse erhalten haben wurden der „frozen section analysis"-Gruppe (FSA) zugeordnet, jene die keine Gefrierschnittanalyse erhalten haben, wurden der „non frozen section analysis"-Gruppe (nonFSA) zugeordnet. Diese beiden Gruppen wurden mit Hilfe von χ^2-Test und Mann–Whitney U-Test bezüglich des positiven Resektionsrandes, der Lokalrezidivrate und des Tumor spezifischen Überlebens verglichen. Für die Analyse der Lokalrezidivrate wurden jene Patienten, die aufgrund eines positiven Schnittrandes sofort nephrektomiert wurden exkludiert. Zusätzlich habe ich innerhalb der „frozen section analysis"-Gruppe die falschen Diagnosen des histologischen Subtyps untersucht. Hier habe ich den χ^2-Test und ANOVAverwendet.
Ergebnisse:567 Patienten (mittleres Alter 61,6 Jahre ± 12 Jahre, 64,6% Männer) unterzogen sich im Zeitraum zwischen 1994 und 2013 einer Nierenteilresektion. Vergleicht man jene Patienten, die während der Operation eine intraoperative Gefrierschnittuntersuchung erhalten haben mit denen die keine Gefrierschnittuntersuchung erhalten haben, findet man keine Unterschiede hinsichtlich Tumorhistologie, Tumorgrad (Fuhrman), Tumorstadium, Gefäßinvasion, sarkomatiode Differenzierung Tumordurchmesser.
Die Gefrierschnitt Resektionsränder waren positiv in 103/442Patienten (23.3%) der „FSA"-Gruppe. Dies führte zu sofortiger Nephrektomie in 75 Fällen, wovon die endgültige

Histologie in 22/75(29%) Nieren Resttumor zeigte, während 53/75(71%) Nieren tumorfrei waren. Somit änderte die Gefrieschnittdiagnostik den Verlauf der Operation bei 75/442 (17%) Eingriffen, darunter in 22/442 (5%) Fällen gerechtfertigt, in 53/442 (12%) ungerechtfertigt.
Die Paraffin Histologie ergab einen positiven Resektionsrand in 7/125 (5.6%) Fällen der „nonFSA"-Gruppe verglichen mit 47/442 Fällen (10.8%) der „FSA"-Gruppe (p = 0.09). Die Gefrierschnittuntersuchung ergab hinsichtlich der Resektionsrandanalyse im Vergleich zur Paraffinhistologie eine Sensitivität von 74.5%, eine Spezifität von 82.8%, einen positiven Vorhersagewert von 34% und einen negativen Vorhersagewert von 96,5%.
Die weitere Analyse hinsichtlich Lokalrezidiven basierte nach Ausschluß der nephrektomierten Patienten auf 367 Nierenteilresektionen in der FSA-Gruppe. Insgesamt 20 Lokalrezidive nach Nierenteilresektion wurden beobachtet, darunter 17/367 (4,6%) Patienten in der „FSA"- Gruppe und 3/125 (2,4%) Patienten in der „nonFSA"-Gruppe (p = 0.33). 5 Patienten mit einem Lokalrezidiv hatten einen positiven und 12 einen negativen Resektionsrand im Gefrierschnitt. Die Kaplan-Meier Analyse der Überlebenskurven zeigte keine signifikanten Vorteile bezüglich des tumorspezifischen Überlebens in der Gruppe, die eine intraoperative Gefrierschnittuntersuchung erhalten hat.
Die Ergebnisse der Gefrierschnittuntersuchung und der endgültigen histopathologischen Untersuchung hinsichtlich der Tumorentitäten stimmten in 90% (400/442) der Fälle überein. Von den 42 Patienten (10%) in denen Gefrierschnitt und Paraffinhistologie nicht übereinstimmten wurden 4 Patienten (10 %) überdiagnostiziert und 38 (90%) wurden unterdiagnostiziert.
Die univariable Analyse konnte keinen Zusammenhang zwischen der Genauigkeit der Gefrierschnittuntersuchung mit dem Patientenalter, Tumorgröße, Vorhandensein und nichtvorhanden sein einer sarkomatoiden Dedifferenzierung, der Lokalisation des Tumors, dem Tumorstadium und dem Tumorgrad zeigen.
Schlussfolgerung: Die Verwendung der Gefreirschnittuntersuchung im Zuge einer Nierenteilresektion beim Nierenzellkarzinom hatte in unserer Analyse keinen Einfluß auf das onkologische Ergebnis hinsichtlich Lokalrezidiv- oder Metastasierungsrate. Der niedrige positive Vorhersagewert von 34% resultierte darüber hinaus in einer Übertherapie in Form unnötiger Nephrektomie bei 12% der Patienten in der Gefirerschnittgruppe, während nur 5% aufgrund von Resttumor im Nephrektomiepräprat profitierten.

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List of Abbreviations

RCC	renal cell carcinoma
HRC	hereditary renal cancer
ETS	environmental tobacco smoke
RR	relative risk
ESRD	end stage renal disease
TCE	trichloroethylenef
OR	odds ratio
CCRCC	clear cell renal cell carcinoma
VHL	von Hippel-Lindau
PFS	progression free survival
HPRCC	hereditary papillary renal cell carcinoma
BHD	Birt-Hogg-Dube‘ syndrome
pVHL	von Hippel-Lindau protein
HIF	hypoxia inducible factor
HPRC	hereditary papillary renal carcinoma
HGF	hepatocyte growth factor
HLRCC	hereditary leiomyomatosis and renal cell cancer
MCUL	called multiple cutaneous and uterine leiomyomatosis
FH	fumarate hydratase
chRCC	chromophobe renal cell carcinoma
AMPK	adenosine monophosphate activated protein kinase
mTOR	mammalian target of rapamycin
TSC	tuberous sclerosis complex
HPT-JT	Hereditary hyperparathyroidism jaw tumor syndrome
CDC73	cell division protein 73
PTC-PRN	papillary thyroid carcinoma with associated papillary renal neoplasia
SDH	succinate dehydrogenase
CTL	cytotoxic T- lymphocyte
MHC	major histocompatibility complex
TGF-β	transforming growth factor β
TAA	tumor associated antigens
HSP	heat shock protein

TAP	transporter associated with antigen presentation
IFN-γ	interferon γ
KIR	Killer-cell immunoglobulin-like receptor
NKC	natural killer cells
CTLA4	Cytotoxic T-Lymphocyte Antigen 4
MDR	multidrug resistance
MRP	multidrug resistance protein
ABC	ATP-binding cassette
ATP	adenosine triphosphate
bFGF	as basic fibroblast growth factor
VEGF	vascular endothelial growth factor
PDGF	platelet-derived growth factor
TKI	tyrosine kinase inhibitors
PFS	progression free survival
EGF	epidermal growth factor
IGF	insulin like growth factor
IGFBP	insulin-like growth factor binding protein
BCL2	B-cell CLL/lymphoma 2
HGF	hepatocyte growth factor
ECM	extracellular matrix
BM	basement membrane
MMP	matrix metalloproteinases
TIMP	tissue inhibitors of matrix metalloproteinase
WHO	World Health Organization
pRCC	papillary renal cell carcinoma
CTG	chromophobe tumor grade
cdRCC	carcinoma of the collection ducts of Bellini
CT	computer tomography
MRI	magnetic resonance imaging
HU	Hounsfield Units
SSING	stage, size, necrosis, grand prognostic system
MSKCC	Memorial Sloan Kettering Cancer Center
CRP	C – reactive protein
UISS	University of California Los Angeles integrated staging system

LPN	laparoscopic partial nephrectomy
OPN	open partial nephrectomy
5-FU	5-flurouracil
DC	dendritic cells
TH1	T-helper cell type 1
LAK	lymphokine activated killer cells
VEGFR-1	vascular endothelial growth factor receptor 1
PD-1	Programmed death 1
PD-L1	Programmed death ligand 1
FKBP	FK binding protein
PI3K	phosphoinositide 3-kinase
AKT	protein kinase B
mTORC1/2	mammalian target of rapamycin complex ½
mLST8	mammalian lethal with SEC13 protein 8
4E-BP1	4E binding protein-1
eIF-4E	eukaryotic initiation factor-4 subunit E
Pro	proline
P	phosphorous
Ub	Ubiquitin
FGF	fibroblast growth factor
IL-8	interleukin-8
MSIN1	mammalian stress–activated protein kinase–interacting protein 1
RTK	receptor tyrosine kinases
PTEN	phosphatase and tensin homologue
P70S6K	P70S6 kinase
OS	overall survival
PFS	progression free survival
EAU	European association of Urology
FS	frozen section
PN	partial nephrectomy
PSM	positive surgical margin

I. Chapter: Introduction to the field

1. Lead-in

1.1. Definition of the renal cell carcinoma (RCC)

Benign and malignant tumors of the kidney originate from the epithelial lining of the tubule system on the one side, and from the mesenchymal tissue parts on the other side. The epithelial component is the most common origin of the renal tumors. (1)
Around 90% of renal tumors are malignant; out of those, 85% are renal cell carcinomas. (2) The renal cell carcinoma was often mistakenly called hypernephroma. The name was given by Paul Grawitz in 1883, when he published his observations on the morphology of small, yellow renal tumors. He concluded that renal tumors originate from the adrenal gland, therefore he called it hypernphroma. Today, the term hypernephroma is obsolete, but in literature, the renal cell carcinoma is still called Grawitz tumor. (3)

1.2. Epidemiology and Etiology

Around 3-4% of all malignant neoplasms in adults are renal neoplasms. In most of these cases (80-90%) the tumor is classified as a renal cell carcinoma, the remaining 10-20% are urothelial cell carcinomas. Other tumors of the kidney, like sarcomas, lymphomas and metastases from other tumors are rare.(4)

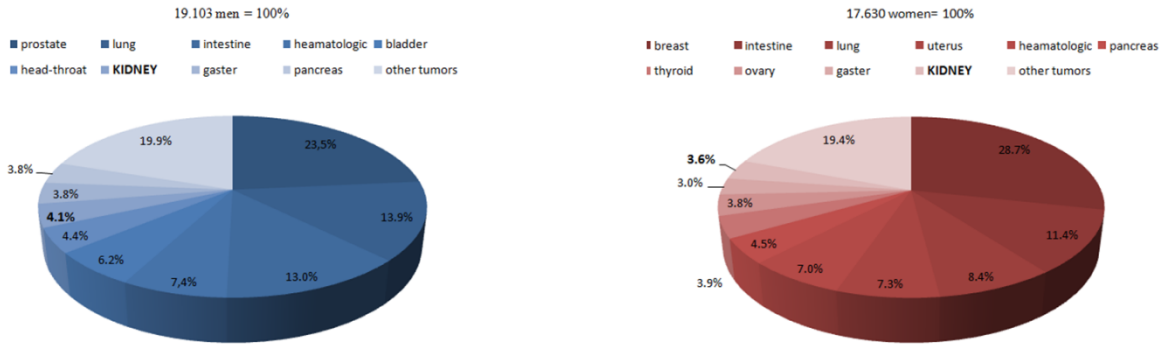

Figure I -1: Distribution of the most common malignant neoplasia
A) Chart A shows the most common malignant tumors in the male Austrian population in the year 2011 and its distribution. In the year 2011, 19103 men were diagnosed with cancer, 4.1% of kidney cancer. (5)
B) Chart B shows the most common malignant tumors in the female Austrian population the year 2011 and its distribution. In women is the incidence of kidney cancer lower than in man. (3.6%) (5)

In 2011, 1243 (age-adjusted: 8.6/100.000 inhabitants/year) patients of the Austrian population were diagnosed with kidney cancer. 61% of them were male. Generally, during the last two decades and until recently, there has been an annual increase of about 2% in the incidence both worldwide and in Europe, although in Denmark and Sweden a continuing decrease has been observed. (6) In 2008, it was estimated that there were 88,400 new cases of RCC and 39,300 kidney cancer-related deaths in the European Union. In Europe, the overall mortality rates for RCC increased up until the early 1990s, with rates generally stabilizing in the following years, but increasing again in recent years. There has been a decrease in the mortality since the 1980s in Scandinavian countries and since the early1990s in France, Germany, Austria, the Netherlands, and Italy. However, in some European countries (Croatia, Estonia, Greece, Ireland, Slovakia), the mortality rates are still showing an upward trend, with increasing rates. (7)

The mortality rate in Europe is 14,500 in females and 24,800 in males (both sexes 39,300) (4). Renal cell carcinoma is the commonest solid lesion in the kidney and accounts for approximately 90% of all kidney malignancies. It includes different types, with specific histopathological and genetic characteristics. (8) There is a 1.5:1.0 predominance of men over women, with the peak incidence occurring between the ages of 60 and 70. Etiological factors include lifestyle factors such as smoking, obesity, and hypertension. (9, 10, 11) Obesity is a controversial issue, as there have been reports showing a better prognosis for obese patients suffering from renal cell cancer. (12) Having a first-degree relative with kidney cancer is also associated with an increased risk of RCC (13, 14).

The most effective prophylaxis is to avoid cigarette smoking and obesity.

As tumors are detected more frequently using imaging techniques such as ultrasound and computed tomography (CT), the numbers of RCCs diagnosed incidentally has increased. These tumors are more often smaller and at a lower stage (14, 15).

2. Forms of the Renal Cell Carcinoma

2.1 Sporadic renal cell carcinoma

The sporadic RCC originates without any germ line mutations and with 96% of all RCC, it is the most common form. The most common histological tumor type of sporadic RCC is the clear cell renal cell carcinoma (CCRCC). The preferred location of this malignant tumor is the renal cortex. (16) From the molecular pathologic view, mutations on the long arm of chromosome 3 seem to be important for the development for sporadic and hereditary RCC, particularly for the von Hippel-Lindau gene (VHL). The VHL-gene is a tumor suppressor gene which is located on chromosome 3q25. (17). It has been shown that VHL-mutations cause up to 57% of sporadic RCCs (18), but the published data is controversial. (19, 20, 21)

At first, the VHL mutations status was thought to be a good prognostic parameter reading clinical outcomes. (22) But, only patients with a loss of function mutation in VHL had a significantly decreased progression free survival (PFS). (23)

2.2. Hereditary renal cell carcinoma

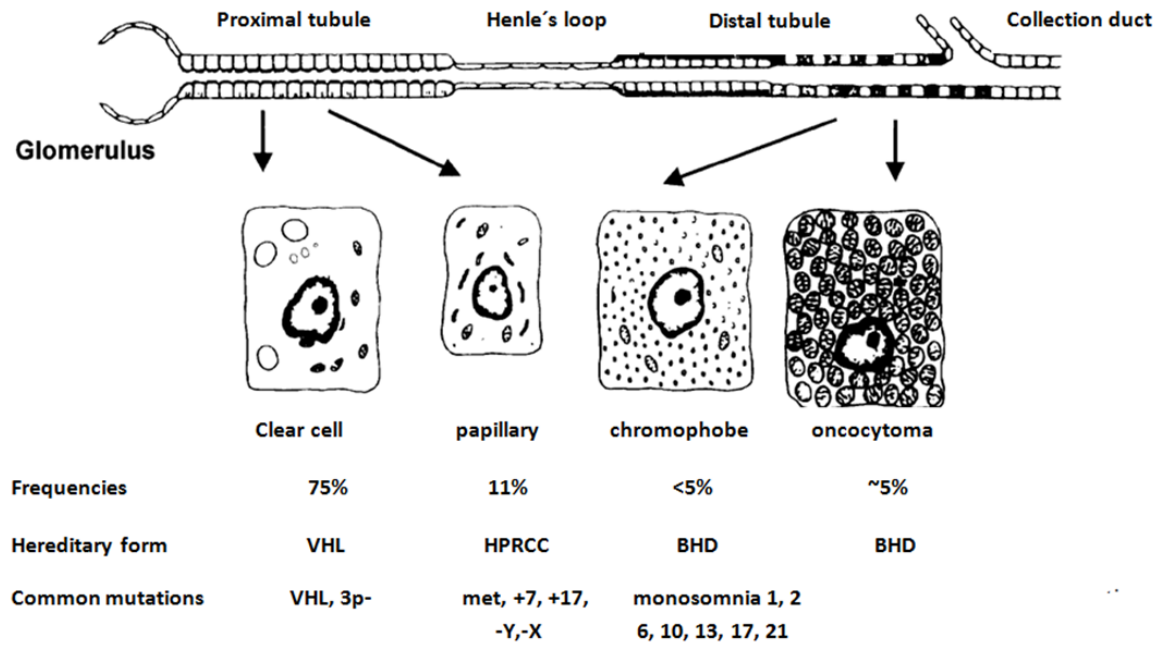

Figure I -2: Origin of RCC in the nephron and correlation with hereditary syndromes
This figure shows the basic histological types of RCC. Clear Cell and papillary (chromophile) RCC origin form the proximal tubule. The chromophobe RCC and the oncocytoma develop in the distal tubule. Hereditary syndromes are specifically associated with the histological types – VHL with the clear cell RCC, hereditary papillary renal cell carcinoma (HPRCC) with the papillary subtype and the Birt-Hogg-Dube-syndrome (BHD) with oncocytoma and chromophobe renal cell carcinoma. If the mutations occur in somatic cells they will develop into the specific histological subtypes – then call sporadic RCC (24)

To date, 10 different hereditary RCC syndromes have been described. The VHL syndrome is the best known hereditary RCC syndrome. (25) Hereditary RCC tends to occur earlier in life than sporadic forms, and often occurs bilateral and multifocally. Unlike sporadic renal cancer, which commonly develops in the 6th and 7th decade, hereditary cancers may develop much earlier in life. (26) Another important areas regarding heritable RCC syndromes are tumor-suppressor genes. In their absence or if they are rendered as nonfunctional due to mutation, tumors can develop, particularly when both copies of the gene are rendered as nonfunctional. The absence or mutation of one copy of a tumor-suppressor gene is commonly not enough to cause a tumorgenesis. It seems that both copies have to be malfunctioning for tumor development. This concept is known as the Knudson hypothesis or the "two-hit" hypothesis. (27) The best known tumors that arise due to this hypothesis are the retinoblastoma and the heritable RCC. (28)

But there are also other important mechanisms, which seem to play an important role in tumor pathogenesis of heritable RCC, like epigenetic mechanisms, malfunctioning of mismatch repair genes and gain of function mutations in proto-oncogene that lead to an uncontrolled production of growth factors and to oncogenesis. (29)

Syndrome	Causative gene, location	Gene product	Renal tumours	Other tumours
VHL disease	*VHL*, 3p25-26	pVHL	Clear cell RCC: solid and/or cystic, multiple, and bilateral Clear cell renal cysts	Retinal and CNS haemangioblastomas, phaeochromocytoma, pancreatic cyst and endocrine tumour, endolymphatic sac tumour, epididymal and broad ligament cystadenomas
Hereditary papillary RCC	*MET*, 7q31	MET	Type 1 papillary RCC: multiple and bilateral	None
Hereditary leiomyomatosis and RCC	*FH*, 1q42-43	Fumarate hydratase	Papillary RCC (non–type 1): solitary and aggressive	Uterine leiomyoma and leiomyosarcoma, Cutaneous leiomyoma and leiomyosarcoma
BHD syndrome	*FLCN (BHD)*, 17p11.2	Folliculin	Hybrid oncocytic RCC, chromophobe RCC, oncocytoma, clear cell RCC: multiple and bilateral	Cutaneous lesions (fibrofolliculoma +++, trichodiscoma, acrochordon), lung cysts, spontaneous pneumothorax, colonic polyps or cancer
Tuberous sclerosis complex	*TSC1*, 9q34	Hamartin	Angiomyolipoma, clear cell RCC, cyst, oncocytoma: bilateral and multiple	Facial angiofibroma, subungual fibroma, hypopigmentation and café au lait spots, cardiac rhabdomyoma, seizure, mental retardation, CNS tubers, lymphangioleiomyomatosis
	TCS2, 16p13.3	Tuberin		
Hereditary hyperparathyroidism-jaw tumour syndrome	*CDC73 (HRPT2)*, 1q24-32	Parafibromin	Papillary RCC, hamartoma, nephroblastoma, cyst	Parathyroid tumour, fibro-osseous mandibular and maxillary tumour, uterine tumour
Papillary thyroid carcinoma with associated papillary renal neoplasia	Unknown gene, 1q21	Unknown	Papillary RCC and adenoma, oncocytoma	Papillary thyroid cancer, nodular thyroid disease
SDHB-associated hereditary paraganglioma/phaeochromocytoma	*SDHB*, 1p36	SDHB	Clear cell RCC	Paraganglioma, phaeochromocytoma
Constitutional chromosome 3 translocations	Unknown gene	Unknown	Clear cell RCC: multiple and bilateral	None
Familial clear cell RCC	Unknown gene	Unknown	Clear cell RCC: solitary	None

Table I- 1: Table shows all known heritable RCC syndromes

Up to now we know about 10 different entities of renal cancer syndromes. They differ in causative gene that is mutated and the histological type of the resulting RCC. From up to down they are ordered towards there prevalence. So von Hippel- Lindau (VHL) is much more common than familial clear cell carcinoma. (30)

2.2.1 von Hippel-Lindau (VHL) disease

The von Hippel-Lindau disease is an autosomal dominant inherited cancer disease and was first described by the German pathologist von Hippel and the Swedish ophthalmologist Lindau. (31) The most common VHL related tumors are clear cell renal cell carcinomas (CCRCC), central nervous tumors, particularly cerebellar, brain stem and retinal hemangioblastomas, pheochromocytomas, pancreatic neuroendocrine tumors, pancreatic cysts, endolymphatic sac tumors and epididymal papillary cystadenomas. (32)

In VHL, the VHL tumor suppressor gene is located and mutated on chromosome 3p25-26. The estimated incidence of VHL ranges from 1:36000 to 1:53000 with a penetrance of up to 95%. It is the most common form all the hereditary RCC syndromes. The genotype-phenotype correlation splits the VHL up into three subtypes. (33) Type 1 VHL often has deletion or nonsense mutation. Type 1 is mostly associated with hemangioblastomas whereas in CCRCC it is rare. Type 2 is subdivided into 2A, 2B, 2C which are mostly characterized by missense mutations. 2A is associated with hemangioblastomas and pheochromocytomas but not CCRCC, 2B is related to hemangioblastomas, pheochromocytomas and CCRCC and 2C has a risk for pheochromocytomas. Type 3 VHL has only a risk of Chuvash polycythaemia and no influence on renal carcinoma. (34)

Renal cell carcinomas are recognized in 24 - 45% of von Hippel-Lindau patients. (35)VHL positive renal masses are potentially malignant and typically low grade and slow growing. The treatment of these lesions should be based on the size of the largest solid part rather than the guidelines proposed sporadic lesions. (36)

The germ line mutation in the VHL gene is accompanied by inactivation of the wild type copy of the VHL gene due to the loss of heterozygosity, promoter hypermethylation or somatic mutation. This is in accordance to the two-hit theory of Knudson for mutations in tumor suppressor genes. (37)

The VHL - gene codes the VHL – protein (pVHL) which forms a multiprotein complex with Cullin 2, Rbx1 (or Roc1), NEDD8, and Elongin B and Elongin C. This complex works as an E3 ubiquitin ligase. The complex targets on the α-subunits of hypoxia inducible factor (HIF), that is, HIF-1α and HIF-2α, for ubiquitin-mediated quarrying which is an oxygen mediated process. Under hypoxia, pVHL is unable to bind HIF-α, so it destroyed by ubiquitination.

Inactivating mutations of both copies of VHL lead to a status which is similar to hypoxia and HIF accumulates. HIF activates the expression of hypoxia-inducible factors like vascular endothelial growth factor (VEGF), glucose transporter 1, platelet derived growth factor-β and transforming growth factor-α and erythropoietin. Each of these factors plays a significant role in tumor pathogeneses. (38)

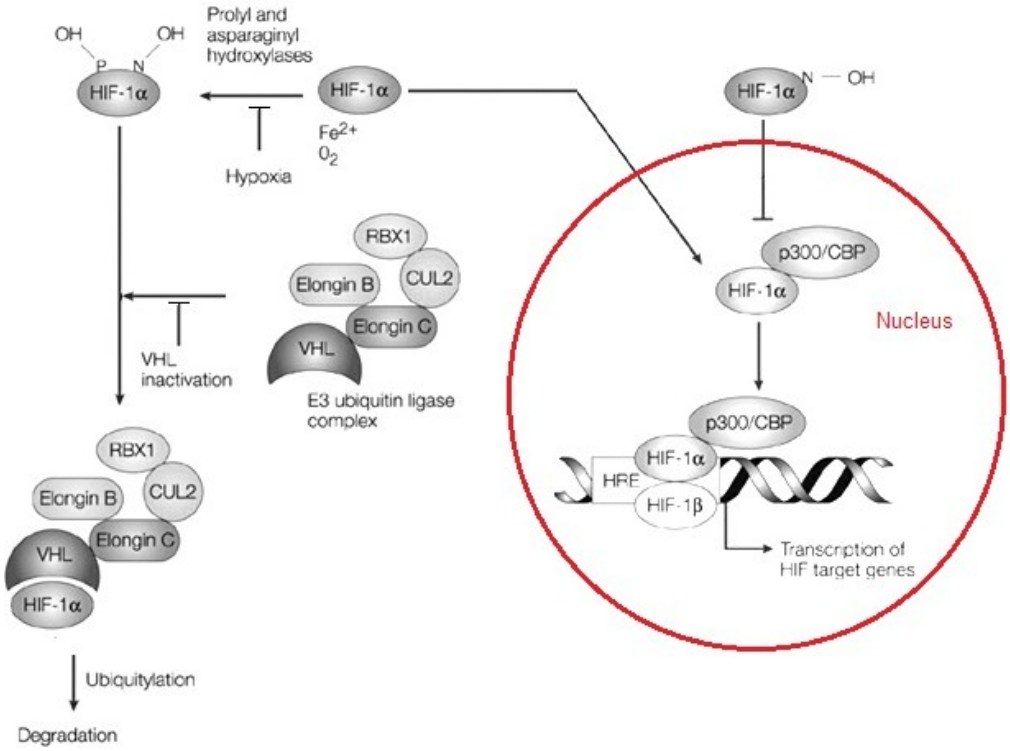

Figure I -3: Shows the molecular mechanisms in VHL

Under normoxic conditions, HIF-1 is hydroxylated. This hydroxylation provides a substrate-recognition site for the VHL - E3 ubiquitin ligase complex. Polyubiquitylation of HIF1- by the VHL complex leads to its proteasomal degradation. HIF1 is also hydroxylated by FIH-1, an asparaginyl hydroxylase. This blocks binding of the transcriptional co-activators CREB-binding protein and p300 to HIF-1, thereby inhibiting transcription of HIF target genes. Hypoxic conditions block both types of hydroxylation, allowing HIF-1 subunits to accumulate and activate transcription of hypoxia-responsive genes. VHL inactivation, as occurs in renal cells from patients with a germ line VHL mutation and loss of the wild-type allele. Loss of VHL function causes accumulation of HIF-1 in the cytoplasm and their translocation to the nucleus. HIF-1 dimerizes with HIF-1 and is co-activated by CBP/p300. HIF binds to hypoxia response elements (HRE) in gene promotors, including vascular endothelial growth factor, erythropoietin and platelet-derived growth factor. (37)

2.2.2 Hereditary papillary renal carcinoma (HPRC)

HPRC is an autosomal dominant inherited disease. It is very rare disease with a typically which occurs in the elderly (between 50 and 70 yrs of age). (39) (40) Papillary renal cell carcinomas are the only type of RCC associated with HPRC. (41) These papillary tumors are well differentiated (Fuhrman grad 1-2) and have a good cancer-specific prognosis, metastatic disease is very rare. (40) The disease is caused by a germ line mutation in the MET gene located on 7q31. MET is a proto-oncogene that encodes for a cell surface receptor called hepatocyte growth factor (HGF) (41)

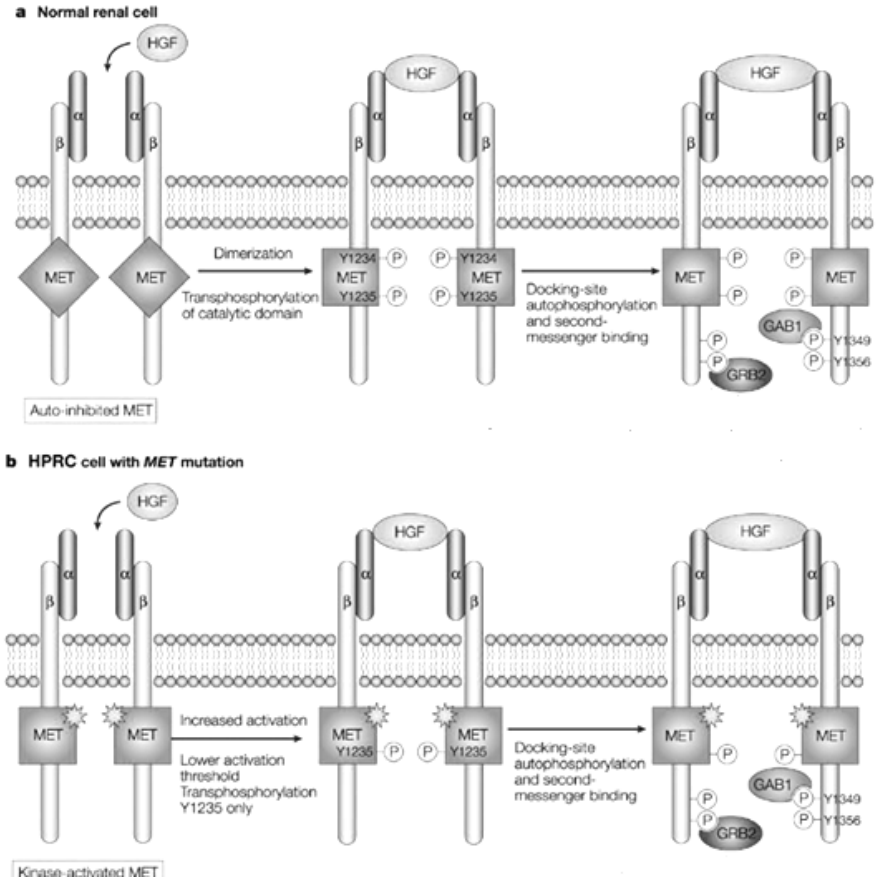

Figure I -4: Molecular pathogenesis of hereditary papillary renal carcinoma (HPRC)
A) In normal cells, HGF binds the MET receptor to induce MET dimerization and release auto-inhibition. This permits transphosphorylation of catalytic tyrosine. Subsequent phosphorylation of multisubstrate docking sites promotes binding of second-messenger molecules, such as GRB2, GAB1 and downstream signaling leading to morphogenic, motogenic and mitogenic programs. **B)** Renal cells from patients with HPRC can harbor germline mutations in the tyrosine kinase domain of MET. These mutations are predicted to release the auto-inhibition by the MET, allowing the receptor to transition to the active kinase form in the absence of a ligand. Signals for proliferation, invasion and survival occur after docking-site phosphorylation and second-messenger binding. (37)

Additional steps such as duplication of mutant MET-bearing chromosome 7 and trisomy of chromosomes 16, 17 and 20 might be necessary for the development of these late-onset papillary renal carcinomas. (42)

2.2.3 Hereditary leiomyomatosis and renal cell cancer (HLRCC)

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is recently identified as a heritable RCC syndrome. HLRCC was first described in 2001. (43) HLRCC is a variant of another syndrome called multiple cutaneous and uterine leiomyomatosis (MCUL), where RCCs occur rarely. Both HLRCC and MCLU are inherited as autosomal-dominant conditions with an incomplete phenotype penetrance. (44) RCC was found in about 20% of HLRCC families with an early onset (mean age: 36-39 years). (45)
Until recently, it was thought that only type 2 papillary RCCs are associated with HLRCC. (46) However, recent publications show that HLRCC tumors displayed a predominant papillary pattern in only 62.5%. (47) In contrast to the other forms of heritable RCC syndromes, HLRCC associated RCCs are solitary, unilateral, and typically high grade tumors (Fuhrman 3-4). (43) HLRCC associated RCCs are the most aggressive renal tumors occurring in heritable RCC syndromes, most patients die within 5 years because of metastatic disease. (48) Also cutaneous and uterine leiomyomas and leiomyosarcomas occur in this syndrome. (43, 46) The causing gene of HLRCC was mapped on chromosome 1q42.3-q43 and corresponds to the fumarate hydratase (FH) gene. Biallelic inactivation was detected in almost all HLRCC tumors, suggesting that FH is a tumor suppressor gene. (49) FH gene is a gene that encodes for fumarate hydratase, which is an enzyme of the Krebs cycle that catalyzes the conversion from fumarate into malate. The complete role of FH in tumor pathogenesis is unclear. It was suggested that fumarate accumulates in the cell and induces HIF-α. (50) Also a role of FH in DNA damage response was suggested. (41)

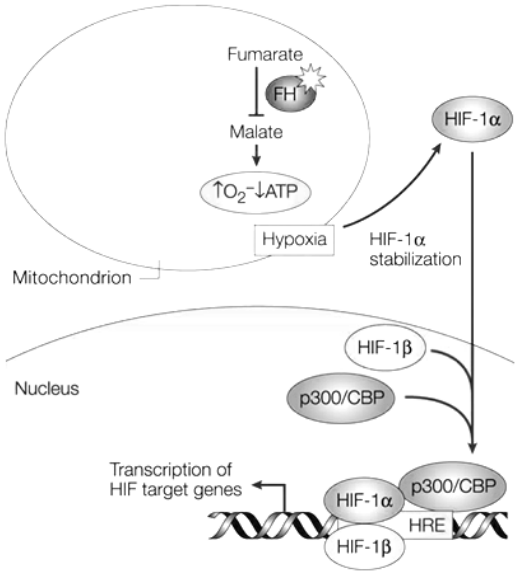

Figure I -5: Showing the molecular mechanism of HLRCC

It is likely that impaired mitochondrial function due to fumarate hydratase (FH)-inactivating mutations which block the conversion of fumarate to malate by the Krebs cycle lead to severe energy deficits (depletion of ATP) and the formation of oxygen free radicals (O2-). This is sensed by the mitochondria as hypoxia, which leads to stabilization of HIF-1 subunits (see figure 3) and transcriptional up-regulation of hypoxia-inducible genes such as vascular endothelial growth factor, erythropoietin, platelet-derived growth factor- and transforming growth factor-106. These proteins promote cell proliferation that could activate tumor growth. (37)

2.2.4 Birt-Hogg-Dube' syndrome (BHD)

The Birt-Hogg-Dube' (BHD) syndrome is a very rare autosomal-dominant genodermatosis, characterized by the development of skin, lung, and kidney lesions. It occurs in about 1:200.000 of the population. BHD has great clinical variability and therefore difficult to diagnose. (51) The risk to develop a renal tumor or a spontaneous pneumothorax is 7-50 fold higher than in the general population. (37)

Major criteria

- At least five fibrofolliculomas or trichodiscomas, at least on histologically confirmed, of adult onset
- Pathogenic FLCN germ line mutation

Minor criteria

- Multiple lung cyst: bilateral basally lung cysts with no other apparent cause, with or without spontaneous primary pneumothorax
- Renal cancer: early onset (< 50 yr of age) or multifocal or bilateral renal cancer of mixed chromophobe and oncocytic histology
- A first degree relative with BHD syndrome

Table I- 2: Diagnostic criteria for BHD-syndrome.
According to Menko et al (59)

In contrast to the other heritable RCC syndromes, BHD associated renal tumors differ in their histological subtypes. Chromophobe RCC (chRCC) and hybrid oncocytic tumors (mixed pattern of chRCC and oncocytomas) are the main subtypes (23% and 67%, respectively); oncocytomas, papillary RCC and ccRCC are very rare. (52) BHD syndrome results from a mutation (germ-line) of the FLCN gene (previously known as BHD gene) located on 17p11.2. (61) The FLCN protein, called folliculin is a tumor suppressor protein involved in the regulation of adenosine monophosphate activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signaling pathway (53,54)

2.2.5 Tuberous sclerosis complex (TSC)

Tuberous sclerosis complex (TSC) is a genetic neurocutaneous disorder characterized by formations of hamartomas in multiple organ systems. The incidence is 1:6000 to 1:10000. The disease has autosomal dominant trait with a nearly complete penetrance with variable expressivity. Renal lesions occur in 50-80% of TSC patients and include angiomyolipomas, cysts, oncocytomas and RCC´s. (55) The overall incidence of RCC approximates that of the general population but it occurs at a younger age (28 years age). (56) TSC results from germ line mutations either in TSC1 (9q34) encoding for harmatin, or TSC2 (16p13.3) and encoding for tuberin. (55,57) Harmartin and tuberin bind to each other and form a complex that inhibits the downstream pathway of mTOR. (58)

2.2.6 Hereditary hyperparathyroidism jaw tumor syndrome (HPT-JT)

Hereditary hyperparathyroidism jaw tumor syndrome (HPT-JT) is a rare autosomal dominant inherited tumor syndrome characterized by predisposition to develop primary hyperparathyroidism, caused by parathyroid adenomas or more rarely parathyroid carcinomas combined with multiple ossifying jaw fibromas. Approximately 15 % of HPT-JT patients develop a renal manifestation of the syndrome including polycystic kidneys, harmartomas, adenomas, RCC, late onset Wilms' tumors. (59, 60)
The HPT-JT gene was identified on 1q24-32 and corresponds to the cell division protein 73 homolog genes (CDC73), which acts as tumor suppressor gene. The protein product from this CDC73 gene works indirectly as inhibitor of the c-myc proto oncogene. (61)

2.2.7 Papillary thyroid carcinoma with associated papillary renal neoplasia

An unusually large three generation family with papillary thyroid carcinoma was described in which two members had papillary neoplasms. The renal lesions were described as papillary RCC, papillary adenomas and oncocytomas. A linkage analysis was performed showing that this PTC-PRN phenotype is linked to 1q21. (62)

2.2.8 SDHB-associated hereditary paraganglioma / phaeochromocytoma

This disease is an autosomal-dominant condition in which affected individuals are at risk for the development of phaeochromocytoma and paraganglioma. SDHB associated hereditary phaeochromocytoma / paraganglioma is caused by a germ line mutation in succinate dehydrogenase (SDH). Oncocytomas, ccRCC and chRCC were described in correlation with SDHB associated hereditary phaeochromocytoma / paraganglioma. (63)

2.2.9 Constitutional chromosome 3 translocations

Constitutional balanced chromosome 3 translocations are an uncommon cause of hereditary ccRCC. 13 different constitutional translocations with ccRCC susceptibility have been described. (64)

2.2.10 Familial clear cell renal cell cancer

Familial ccRCC is defined by the development of ccRCC in two or more family members and no evidence of the ccRCC susceptibility syndrome as VHL-syndrome and constitutional chromosome. In the literature, there have been more than 70 families described (65)

These families may represent evidence of a multigenic inheritance mechanism. (37)

3. *Pathogenesis of the renal cell carcinoma*

3.1 Influence of the immune system

The cellular immune response against tumors is rarely efficient in most tumors. (66) Cytotoxic T-Lymphocytes (CTL) are able to recognize and lyse human tumor cells, but this tumor specific CTL were only found in a few patients with tumors and then they have only a marginal lytic potential and a small proliferation rate. (67) The reason for this reduced antigenicity and immunogenicity and/ or functional alteration of the T-cell response, respectively, are based on different mechanisms. These mechanisms are responsible for the ineffective immunologic antitumor effect and include:

- missing or down regulated expression of classic major histocompatibility complex (MHC)
- a deficient MHC class-1and class-2 antigen processing and presentation
- constitutive expression of the not classic MHC-molecules like HLA-G
- expression of CD95 or down regulation of Fas-L
- a missing co-simulation as well as secretion of immunosuppressive factors like transforming growth factor β (TGF-β), prostaglandins and IL-10 (68)

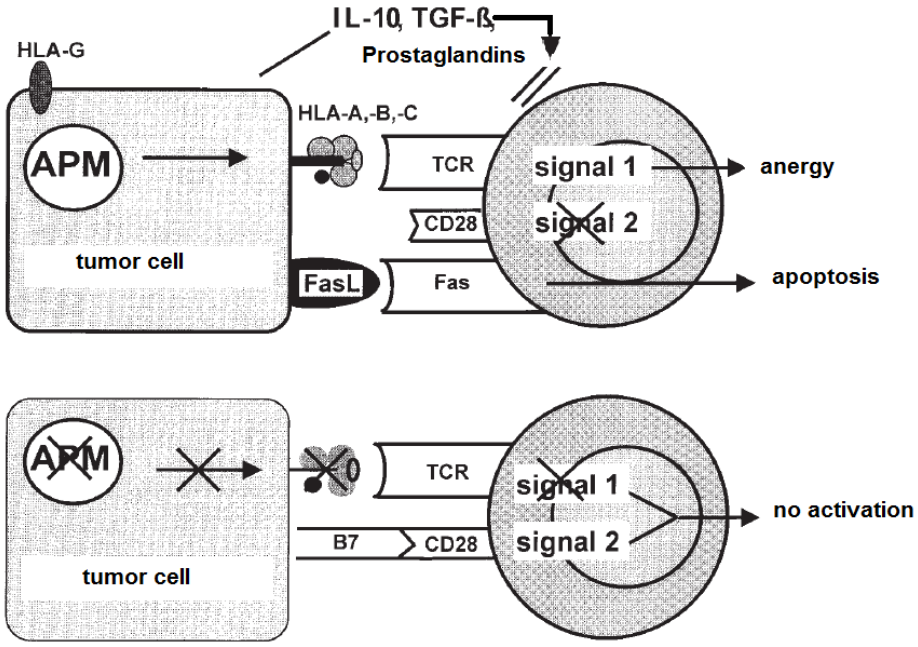

Figure I -6: Immune escape mechanisms of the RCC

In this figure are all known immune escape mechanisms shown that are important for RCC (68)

Like melanoma, RCC represents an immunogenic tumor which is based on the response rate to immunotherapies (IL-2, IFN α/β), the occurrence of spontaneous regressions and the high levels of tumor T cell infiltration. (69, 70)

3.1.1 Expression of tumor associated antigens

Human tumors express antigens which are presented to the CD4+ and CD8+ T-lymphocytes due the MHC-1 and MHC-2. (71) A lot of such tumor associated antigens (TAA) could be identified in RCC like the mutated HLA-A2antigen (72), a mutated form of the stress induced heat shock protein (HSP) 70-2 (73), the family of RAGE antigens, PRAME, MUC-1, gp47, CA-9 (74). These antigens may provide as attractive target for new approaches in immunotherapy like vaccination. In the year of 2012, a vaccination phase 3study with a vaccine called IMA 90 (including a lot of TAA) was published and it's results seems to be very promising, but final results are lacking. (75)

3.1.2 Abnormalities of MHC-1 and inefficient antigen processing

Despite the expression of TAA from RCC, the recognition is caused by the immune system. An essential immune escape mechanism is a reduced antigen presentation due to the MHC-1.This is associated with a reduced MHC-1 surface expression and/or down regulation of different components of the MHC -1 presentation pathway like the peptide transporter complex transporter, associated with antigen presentation (TAP1/2) and the proteasome subunits LMP2/7. (76, 77) The deficient expression of TAP / LMP – subunits, which is unattached to the subtype of RCC, however, correlates with tumor stage and Fuhrman grade, and can be induced due interferon γ (IFN-γ) in most cases. (78) The loss of this IFN-γ mediated induction of TAP/LMP in RCC cell lines is based on a deficiency of the IFN-γ signal transduction pathway. (79)

3.1.3 Aberrant expression of non-classical HLA-antigens

The non-classical HLA-G molecule is an important mediator of immune response. (80) This HLA-G molecule interacts with the Killer-cell immunoglobulin-like receptor (KIR) which is located on natural killer cells (NKC) and CTL and blocks the cytotoxic activity of these NKC and CTL. (81) Thirty percent of examined RCC cell lines express HLA-G constitutively on the cell surface. HLA-G expression of RCC leads to impaired recognition of immune cells allowing RCC cells to escape innate and adaptive anti-tumor responses, which has to be considered for T cell- and NK cell-based immunotherapies. (82)

3.1.4 The role of the Fas/FasL – system

The induction of apoptosis in effector-cells could also be a possible reason for the The induction of apoptosis in effector-cells could also be a possible reason for the ineffective immune response and resistance against chemotherapies in RCC. This effect is mainly mediated by the CD95/CD95L (Fas/FasL) system. Progressive tumors actually express high levels of FasL, which cause the development of an “immune privileged area”. Renal epithelial cells, whichexpress both Fas and FasL during corresponding RCC cell, show a reduced expression of Fas. (83) In contrast to these results Gerharz et al.showed, that CD95L expression is down regulated and CD95 is highly expressed in RCC. The latter has the effect of resistance to the CD95 mediated apoptosis and promotes the evasion of negative growth control. (84)

3.1.5 The role of co-stimulatory factors

The balances between positive and inhibitory co-stimulatory factors play a critical role both in the induction of an effective immune response and the maintaining of the T-cell homeostasis. One of the best known co-stimulatory signals is the B7/ CD28/ CTLA4 system. (85) CD28 is a receptor, which is located on the surface of T-cells. CD28 is able to enhance the tumor specific T-cell response (tumor attack). Cytotoxic T-Lymphocyte Antigen 4 (CTLA4 or CD152) is also expressed on the surface of T-cells and enhances the immunologic tolerance against the tumor. Therefore the T cell attack can be turned on by stimulating the CD28 receptor on the T cell. The T cell attack can be turned off by stimulating the CTLA4 receptor, which acts as an "off" switch.

There are two major types of B7 proteins: B7-1 or CD80, and B7-2 or CD86. However, it is not known if they differ significantly from each other. CD28 and CTLA-4 each interact with both B7-1 and B7-2. The block of the interaction between B7-1 and -2 leads to an enhanced antitumor- immune response. (86, 87, 88)

3.1.6 Expression of immunosuppressive factors

Despite the reinforced T-cell infiltrations, RCC progress and metastasize. A possible explanation could be the secretion of immunosuppressive factors like IL-10, prostaglandins and TGF-β by the RCC tumor cells, apoptotic tumor cells or peripheral lymphocytes. (89) These immunosuppressive factors inhibit an effective antigen presentation and the activation and proliferation cytotoxic T-lymphocytes. (90)

3.2 Multidrug resistance (MDR)

RCC is a chemo and radio resistant tumor, usually showing only a marginal response. (91)The reasons for this resistance could be caused by MDR-1 and the multi-drug resistance protein (MRP). Both MDR-1(ABCB-1) and MRP (ABCC-1) have been demonstrated to pump a wide variety of the most commonly used cancer drugs out of tumor cells. The overexpression of MDR and MRP correlates with the drug resistance in many different human tumors, including pancreatic cancer (92), lung cancer (93) and breast cancer (94). MDR-1 is a transmembrane receptor, which is highly expressed in tumors and develops from tissues with a high density of this surface protein like pancreatic cells, bile duct cells, glial cells and cells of proximal tubule. MDR is an ATP-binding cassette (ABC) transporter. ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair. (95) MDR-1 transports cytotoxic agents such as etoposide, doxorubicin, and vinblastine across the cell membrane and prevents that the agents achieve effective concentrations in the RCC cell. (96)

Also in tumors without increased expression of MDR, the resistance to chemotherapeutics is striking. Anaplastic tumors and metastases show a significant lower expression of MDR on the cells surface in comparison to higher differentiated tumors and the primary

malignant tumors. Therefore, MDR-1 and MRP seem to be not the only mechanisms in the development of chemo-resistance. (97)

3.3 Angiogenesis

A malignant tumor contains a population of rapid growing and mutated cells. Tumors are not able to grow beyond a volume of 1-2mm³ by virtue of a lack of essential nutrients and oxygen. (98)The majority of tumors, including the RCC, depend on the recruitment of blood vessels to enable the tumor growing beyond this little size. Tumors which are unable to induce successful angiogenesis followed by the growth of functional blood vessels remain microscopic in size. (99) Tumor cells in avascular dormant tumors typically exhibit a high proliferation rate that is balanced by elevated apoptosis. Tumors will remain dormant in the non-vascular state until the tumor acquires mutations or otherwise induce the "angiogenic switch" and become angiogenic and rapidly growing. (100) Tumors induce blood vessel growth due to the expression of different growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). These factors can induce capillary growth into the tumor mass. (101) High angiogenesis activity in RCCs is mostly mediated by mutation or epigenetic inactivation of the von-Hippel-Lindau (VHL) tumor suppressor gene and subsequent up-regulation of hypoxia-inducible factor (HIF) expression. (102) Overexpression of HIF results in an increased expression of VEGF and PDGF – and both factors are key players in the development of ccRCC (103)

Clinical trials over the last few years have shown that multiple drugs effectively blocking the angiogenic pathway are very efficient; these agents include tyrosine kinase inhibitors (TKI) [such as sunitinib, sorafenib, axitinib and pazopanib], the anti-VEGF monoclonal antibody bevacizumab (administered with IFN-α) and the mammalian target of rapamycin (mTOR) inhibitors-Everolimus and temsirolimus. (104) Each of these anti-angiogenic drugs provide significant benefit, determined by the rate of objective responses, reduction of tumor burden and extension of progression free survival compared with either immunotherapy or placebo.
Two of the most important studies in the "post cytokine era", Sorafenib showed a prolonged progression free survival [5.5 vs 2.8 month]compared to placebo in cytokine refractory patients in the so called TARGET-trail (double-blinded, randomized). (105)

Sunitinib has also shown a significant prolonged progression free survival (PFS) [11 vs 5 month] compared with IFN-α therapies in untreated patients with ccRCC. This trial further demonstrated a significant prolonged overall survival [26.4 vs 21.8 month]. (106) Despite the therapeutic progress, complete and durable responses have been only noted in a few cases (107), requiring chronic therapies for the vast majority of RCC patients which is often associated with toxicity. (105, 106) Heng et al. showed us that 26% out of 1056 were primary refractory to TKI therapy with sorafenib and sunitinib showing neither disease stabilization nor clinical benefit. These TKI refractory patients also have a poor outcome. (108) While some patients with metastatic RCC are primarily refractory to anti-angiogenic medication, the majority primarily responding to therapy often develop a secondary resistance to certain VEGF target agents typically after 6 to 12 month. (109) The following figure shows the molecular mechanisms of TKI-resistance. (110)

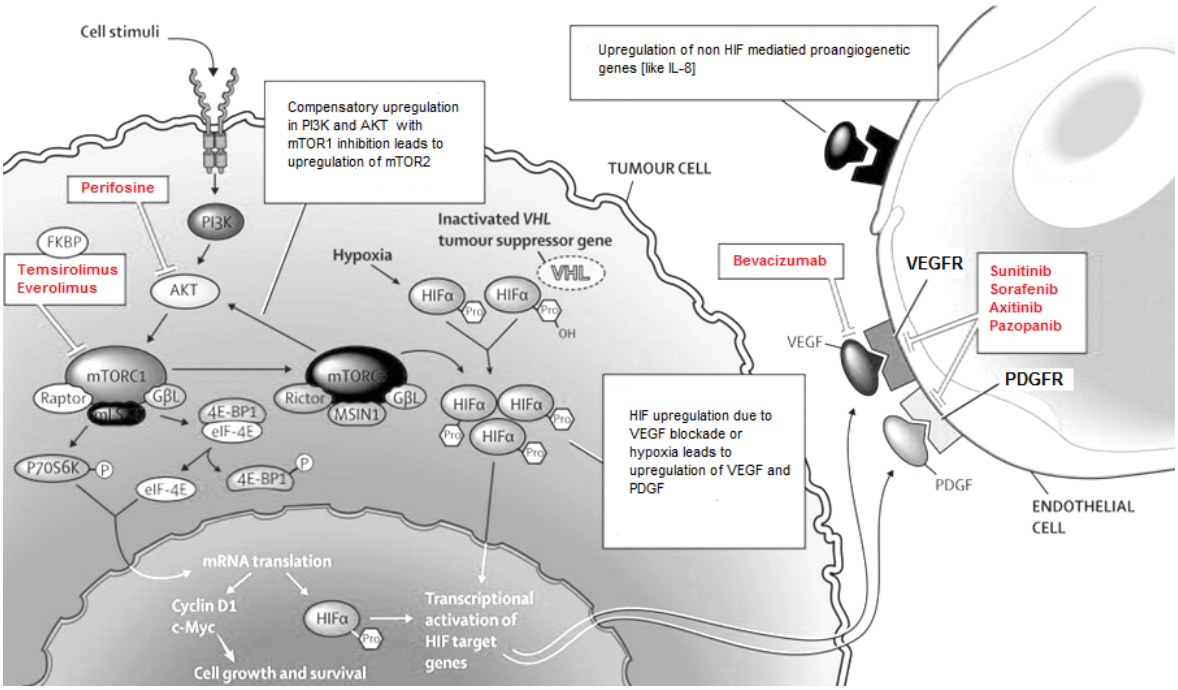

Figure I -7: Molecular pathways in TKI-resistant RCC.

This figure shows the molecular mechanism of TKI resistance.Resistance to existing VEGF-pathway and mTOR-pathway blocking agents can include up-regulation of mTORC2, up regulation of HIF with downstream effects on proangiogenic signaling molecules, activation of alternative angiogenic pathways, or ineffective target inhibition **A)** Acquired resistance: Angiogenic switch – activation of alternative pathways supporting angiogenesis; increased pericyte coverage of tumor vessels; Recruitment of pro angiogenic inflammatory cells from bone marrow; Lysosomal sequestration of sunitinib

B) Intrinsic resistance: Immunomodulatory effect; Apoptosis (110) (170)

3.4 Regulation of the cell cycle and growth-factors in RCC

The regulation and dysregulation of the cell cycle is an important factor in the pathogenesis of renal cancer. (111) Epidermal growth factor receptor (EGFR) and its ligand EGF play an essential role in the control of both normal and malignant cell growth. Increased EGFR has been described in a lot of different malignancies and has been proposed as negative prognostic factor. (112) In RCC frequencies of increased EGFR expression have been reported between 50 – 90%. (113, 114)
An association between increased EGFR expression and high tumor grade and stage has been described. (113) In a mouse model, the chimeric anti-EGF receptor monoclonal antibody C225inhibited the growth and metastasis of RCC. (115)
The epidermal growth factor receptor is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). (112) RCC tumor cells also express the proto-oncogene ErbB-2 which effect is similar to the ErbB-1 (known as EGRR). The over-expression of both receptors is particularly associated with metastatic and advanced RCC. (116) ErbB-2 is an important target for drugs against collecting duct carcinomas of the kidney (also known as Bellini´s Duct carcinomas), one of the most aggressive tumors described in humans. (117) A case report from 2012 showed that a double blockade of ErbB-2 with intravenous Trastuzumab and oral Lapatinib application could improve the clinical response and a progressive decline in the radiological size of all of his multiple cancer lesions, in a patient with disseminated collecting duct carcinoma. (118)

Other important growth factors in RCC are insulin like growth factor (IGF) receptors such as IGF-IR insulin-like growth factor binding protein3 (IGFBP-3).
It has been reported, that IGF-I receptor positive RCC patients experience significantly decreased cancer specific survival and significant higher tumor grades compared to those with IGF-IR negative patients. IGF-IR positive patients have a 70% increased risk of death due to CC-RCC than IGF-IR negative patients (119, 120)
IGFBP-3 is another gene, which is overexpressed in ccRCC. High grade (Fuhrman grades 3 and 4) ccRCCs show a higher IGFBP-3 staining intensity than low grade ones. (121)

The oncogene MYC is differentially expressed in many malignant tumors and plays important role in tumorigenesis. (122) MYC is able to bind specific DNA sequences and functions as a transcription factor to influence the expression of a broad range of human genes involved in cell carcinogenesis, such as cell cycle progression, cell growth, metabolism, apoptosis, and angiogenesis. (123, 124) For example, the MYC-target gene cyclin D1, which is considered as a proto-oncogene, promotes the G1/S phase transition in the progression of cell cycle by regulating the cyclin-dependent kinase activity (125). Another MYC-target gene B-cell CLL/lymphoma 2 (BCL2) inhibits the mitochondrial apoptosis signals (126). Beside these two MYC target genes were 37 different genes described to be a target of MYC and the up-regulation of the MYC-target genes, such as BCL2, CCND1, PCNA, PGK1, and VEGFA. These results suggest that MYC is activated in RCCs and play an important role in the pathogenesis. (127)

Finally, the hepatocyte growth factor (HGF) and the *met* – proto-oncogene pathway seem to play important roles regarding the influence on the cell cycle. The protein product of the *met*-gene is called *c-met* and works as a transmembrane receptor tyrosine kinase. It´s only known ligand is the HGF. HGF regulates cell growth, motility, migration, invasion, proliferation, and angiogenesis. Dysregulation of c-Met and hepatocyte growth factor have been observed in both ccRCC and non-clear cell RCC. In ccRCC, there is evidence of a direct link between loss of VHL and up-regulation of *c-met*. In RCC, a high expression of *c-met* is associated with poor outcome. In vitro and in vivo preclinical RCC models demonstrate cancer control with small molecule and antibodies against c-Met. So there are maybe new therapeutically usable targets in this pathway. (128)

3.5 RCC and the extracellular matrix – tumor adherence

The extracellular matrix (ECM) influences a variety of epithelial cell behaviors such as differentiation, proliferation and morphogenesis. The extracellular matrix is divided into the interstitial matrix and the basement membrane (BM). In carcinomas, RCCs, these properties and interactions of cells with the interstitial matrix and the basement-membrane are disturbed. (129)

Fibronectin is an extracellular glycoprotein that binds and signals due to cell surface proteins known as integrins. (130) The loss of fibronectin - matrix assembly has been recognized to result in malignant transformation. (131)In normal cells, pVHL interacts with fibronectin and forms an extracellular matrix. As known, VHL mutation localized on chromosome 3 is one of the most common mutations in sporadic and hereditary RCCs and causes a disturbed assembly of the ECM.(132) This effect of the VHL- gene shows an essential effect on tumor invasion and metastasis. (133)

Metastases in RCC depend on several factors, such as attachment of malignant cells to the vascular endothelium, penetration through the basement membrane, colonization and proliferation in distant organs. (134) Proteolysis degradation of the basement membrane is a fundamental aspect of cancer development. (135)This degeneration is closely related to the activity of matrix metalloproteinase (MMP) and tissue inhibitors of matrix metalloproteinase (TIMP). It has been shown, that the expression of MMP including MMP-2, MMP-9 and MT1-MMP, correlates significantly with the TNM stage of renal cancer. (136)

Cadherins play important roles in cell adhesion, forming adherend junctions to bind cells within tissues. (137) Loss or abnormal expression of cadherins in tumors influences the progression of the disease. The weak expression of E-cadherin (epidermal cadherin) was identified as significant factor associated with recurrence-free survival. (138) The loss of cadherin -6 was also identified as negative prognostic factor in RCC. It is associated with high grade (Fuhrman 3 and 4) and poor prognosis, respectively. (139)

4. Pathology of renal cancer

4.1 Macroscopic pathology

In macroscopic pathology, RCC is typically located on the poles of the kidney and looks like a single oval to round node. The macroscopic aspect of RCC shows light-yellow to gray-white areas with hemorrhage, necrosis and cysts. These morphological features result in the characteristic “colorful”-picture of renal cell carcinoma. Renal cell carcinomas are often separated from the adjacent parenchyma of the kidney by a pseudo-capsule. Bigger carcinomas are able to infiltrate the perirenal fat, the pelvis of the kidney or the renal vein. Occasionally, RCC develop tumor thrombi which may extend from the inferior vena cava to the right atrium of the heart. (140)

4.2 Microscopic pathology

The final histological diagnosis is performed after surgical removal of the renal tumor.

4.2.1 Major histological subtypes

According to the World Health Organization (WHO), there are three major histological subtypes of renal cell carcinomas. (141)

4.2.1.1 Clear cell renal cell carcinoma (ccRCC)

ccRCC is the most common histological subtype with a percentage of 80-90% of all renal cell carcinomas. (141)
The histological picture of most ccRCC consists predominantly of cells containing clear cytoplasm, although eosinophilic cytoplasm predominates in some cells. The cytoplasm is commonly filled with lipids and glycogen, which are dissolved in routine histologic processing. This process creates the characteristic clear cytoplasm surrounded by a cell membrane. Therefore, the clear cell look is only an artifact of histological preparation. (140) Sarcomatoid differentiation occurs in 5% of RCC and is associated with worse outcomes. (142) Sarcomatoid variants of RCC were described for nearly all histological subtypes but show no de-novo genesis. However, the sarcomatoid differentiation of RCC represents a specific manifestation of high grade RCCs, which can arise from all other RCC subtypes. (143) In general, ccRCC metastasizes along the inferior vena cava

primarily to the lung, although lymphatic metastases could occur. Retrograde metastases via the paravertebral veins, the testicular/ ovary vein and along the ureter occur rarely. ccRCC is well known for its inclination to metastasize to unusual sites and late onset of metastases (after 10 years). (141)

4.2.1.2 Papillary renal cell carcinoma (pRCC)

Papillary renal cell carcinoma (pRCC) comprises about 10-15% of RCCs. (144)
Most pRCC have small cells with sparse cytoplasm, but show also eosinophilic or basophilic characteristics. (141) pRCC is also characterized by forming tumor cells of varying shapes of papillae or tubules. (145) pRCC is further divided into two different subtypes: type 1, with small cell and scanty cytoplasm and type 2, with large eosinophilic cells, which are associated with worse outcomes. (146) Sarcomatoid differentiation is seen in about 5% of pRCC and is associated with both type 1 and type 2. (145)
The Fuhrman nuclear grading system is accepted as applicable grading system in pRCC. (141)

4.2.1.3 Chromophobe renal cell carcinoma (chRCC)

Chromophobe renal cell carcinoma chRCC comprises a percentage of 4-5% of allRCC. (147) In general, the growth pattern of chRCC is solid, sometimes glandular with calcifications and fibrotic septa. chRCC is characterized by large polygonal cells with transparent cytoplasm. These cells are typically mixed with small eosinophilic granulated cells. The eosinophilic variant is only composed of intensively eosinophilic stained cells with prominent membranes. (148) Sarcomatoid dedifferentiation is rare in chRCC. (149)

ChRCC has the best prognosis of these three major histological subtypes. The majority of these tumors are stage T1 and T2 (86%) whereas only 10% show extensive growth, lung metastasis are rare. (152)
The nuclear grading proposed by Fuhrman is currently the most common grading system for RCC. The Fuhrman nuclear grade evaluatesnuclear size, shape or outline, and nucleolar prominenceto stratify RCCs into a 4-tiered grading scheme. (150)

It is controversial whether the Fuhrman nuclear grade of chromophobe RCC has prognostic utility. The problem of the Fuhrman system is that irregular nuclei, prominent nucleoli, and

nuclear pleomorphism are inherently present in chRCC, hence the most chRCC were classified as Fuhrman grade 3 (74%). Therefore, the Fuhrman grading is higher even though the majority of these tumors have an affirmative outcome. So Paner et al. proposed the utility of a novel tumor grading scheme for chRCC called chromophobe tumor grade (CTG). Chromophobe tumor grade rather seems to be a better independent predictor of adverse outcomes than Fuhrman nuclear grade. This novel chRCC grading system will also potentially help stratify patients who are greater at risk of disease progression. (151)

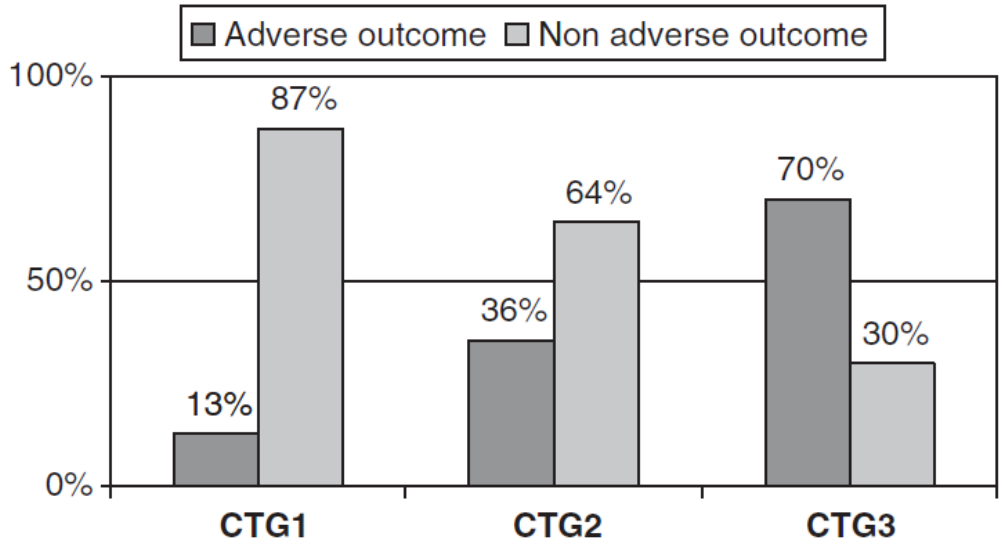

Figure I -8: Association of the novel CTG with adverse outcome
This figure shows the distribution of adverse/ non adverse outcome and the Chromophobe tumor grade in patients with chRCC. According to Paner at al. (151)

4.2.2 Other important histological subtypes

4.2.2.1 Carcinoma of the collection ducts of Bellini (cdRCC)

Collecting duct carcinomas are very rare, but aggressive types of RCC. Collecting duct carcinomas often present at an advanced stage of disease. About 40% of patients are diagnosed with primary metastatic disease. Most patients die within 1-3 years from the time of diagnosis. The hazard ratio regarding cancer specific survival is compared with ccRCC. (4,49) This parameter shows the aggressiveness of this tumor. (153) A large case series showed that 44% of the patients with collecting duct carcinomas present with lymph node metastases and 32% have distant metastases at time of diagnosis. (154) Median survival is 30 months, respectively. (155)

4.2.2.2 Multilocular cystic renal cell carcinoma (mcRCC)

This subtype accounts for up to 2% of surgically treated kidney tumors. (156, 157) Multilocular cystic RCC (mcRCC) is an independent entity of renal cancers, but presents a grade 1 (Fuhrman-grade) clear cell renal cell carcinoma. (158)To date, metastases of this tumor have not been described. (159) This variant of ccRCC has a favorable prognosis. One small sample size study showed that all patients were alive with no evidence of disease at a mean follow-up of 66.1 months, confirming an extremely good prognosis after surgery and a five year tumor-specific survival rate of 100%. These authors also suggest to change the term mcRCC to "multilocular cystic renal cell neoplasm of low malignant potential". (163)

According to the Bosniak-classification, most cases of mcRCCs correspond to category II or III, but even category IV may occur in some cases. (160) However, this type of Bosniak cysts can also be due to other malignant or benign lesions. Multilocular cystic RCC should be distinguished from other renal tumors, including ccRCC, cystic nephroma, mixed epithelial stromal tumors or multilocular cysts. (158)

In most cases, preoperative biopsy or intra-operative frozen section analyses fail to reveal the right diagnosis. Fortunately, all these differential diagnoses are treated with the same operative strategy. (161)

When mcRCC is suspected, nephron sparing surgery should be performed whatever its size and when technically practicable and is the treatment of choice. (162, 160)

There are no strict histopathological criteria for diagnosing mcRCC in the WHO 2004 classification. The cysts are usually lined by a single layer of epithelial cells. Occasionally, the lining consists of more than one layer of cells or a few papillae are present. (141)

4.2.2.3 Oncocytoma

Renal oncocytoma comprises about 3-7% of all renal tumors. (164)
Imaging alone is unreliable to differentiate between the benign oncocytoma and the malignant RCC. Histopathology is the golden standard to diagnose oncocytomas. (165)
The only pre-operative way to render a correct diagnosis is to perform a percutaneous kidney biopsy. However, kidney biopsies have a low specificity for oncocytomas, because oncocytic cells were found in different malignant lesions, such as granular cell variants of RCC and the type 2 pRCC. The alternative management comprises partial nephrectomy or "watchful waiting". However, watchful waiting could be a risky decision, because growth rates from oncocytomas are similar to RCC. (166)

The histological picture of oncocytomas is characteristic. Oncocytomas have solid compact nests, acini, tubules or microcysts. The predominant cell type is the so called oncocyte. The oncocyte has a round to polygonal cell shape with granular eosinophilic cytoplasm, round and regular nuclei with evenly dispersed chromatin, and a centrally placed nucleolus. (141)

4.2.2.4 Papillary adenoma of the kidney

Papillary adenomas are tumors with papillary or tubular architecture and are at maximum 5mm in diameter. Autopsy studies revealed that papillary adenomas increase from 10% (patients younger than 40 years) up to 40% (patients older than 70 years). (141)

4.2.2.5 Renal cell carcinoma, unclassified

Unclassified RCC is a diagnostic category for RCC that cannot be assigned to any other category of RCC –type (141)

4.2.2.6 Angiomyolipoma

Angiomyolipoma (AML) is a benign mesenchymal tumor composed of a variable proportion of adipose tissue, spindle and epithelioid smooth muscle cells, and abnormal thick-walled blood vessels. It can occur sporadically, and is four times more likely in women. It also occurs in tuberous sclerosis (TS), when it is multiple, bilateral, larger, and likely to cause spontaneous hemorrhage. It accounts for approximately 1% of surgically removed tumors. Ultrasound, CT, and MRI often lead to diagnosis due to the presence of adipose tissue. Biopsy is rarely useful. Pre-operatively, it may be difficult to differentiate between tumors composed predominantly of smooth muscle cells and epithelial tumors.

AML can be found in TS in lymph nodes, which is not metastatic disease, but disease with a multicentric genesis. AML can be due to angiotropic-type growth involved in the renal vein even the inferior vena cava. AML with involvement of lymph nodes and tumor thrombus is benign. Only epithelioid AML is a potentially malignant variant of AML. (265,167) AML is associated with a slow and consistent growth rate (<0.1cm/year), and typically has minimal morbidity (168). The main complications of renal AML are retroperitoneal bleeding or bleeding into the urinary collection system, which can be life-threatening. The bleeding tendency is related to the angiogenic component of the tumor that includes irregular and aneurysmatic blood vessels. The major risk factors for bleeding are tumor size, grade of the angiogenic component of the tumor, and the presence of tuberous sclerosis. (169) Primary indications for intervention include symptoms such as pain, bleeding, or suspected malignancy. Most cases of AML can be managed by conservative nephron-sparing approaches, although some cases of AML may require complete nephrectomy. Alternatively, selective arterial embolisation (SAE) and radiofrequency ablation (RFA) can be used. Although SAE is effective at controlling hemorrhage in the acute setting, it has limited value in the longer-term management of AML.

Clinical trials of medical management with m-TOR inhibitors are ongoing and Sirolimus can be combined with deferred surgery.(168,169)

5. *Diagnosis of renal cancer*

5.1 Symptoms and paraneoplastic syndromes

Many renal masses remain asymptomatic until they reach a late stage or big expansion. Currently, more than 70% of renal masses are detected incidentally by imaging including ultrasound, computerized tomography or magnetic resonance imaging to investigate a variety of nonspecific symptoms and other abdominal diseases. (2)
The classic triad of flank pain, gross haematuria, and palpable abdominal mass is now rare (6-10%) and correlates with aggressive histology and advanced disease (171)The wide spread use of imaging allows us to detect earlier stages of RCC. (2)

Paraneoplastic syndromes are very common findings in patients with RCC. Anemia (~52% of cases), hematuria (~ 35%) and hepatic dysfunction (31%) are the most common syndromes. Some of the paraneoplastic syndromes could be used as independent predictors for survival. Cachexia, defined as hypoalbuminemia (less than 3.6mg/dl), weight loss (greater than 5lbs), anorexia or malaise predicts worse disease specific survival. (172)

	% (No./total No.)	Localized/ Metastatic Ratio	HR	p Value (univariate)
Anemia	52.1 (396/760)	0.7	2.0	<0.0001
All hematuria types	35.2 (358/1,016)	0.8	1.2	0.1852
Gross hematuria	24.3 (247/1,016)	0.7	1.2	0.0744
Hepatic dysfunction	31.5 (167/531)	0.6	2.1	<0.0001
Wt loss	22.9 (232/1,011)	0.3	3.0	<0.0001
Malaise	19.1 (192/1,005)	0.6	2.9	<0.0001
Hypoalbuminemia	19.9 (105/528)	0.6	2.3	<0.0001
Flank pain	19.5 (197/1,010)	0.6	1.3	0.0631
Hypercalcemia	13.0 (61/470)	0.6	1.8	0.0223
Anorexia	10.6 (106/1,004)	0.4	3.1	<0.0001
Thrombocytosis	9.2 (60/654)	0.6	2.6	<0.0001
Night sweats	8.4 (85/1,009)	0.6	2.5	<0.0001
Fever	7.8 (79/1,016)	0.5	2.0	<0.0001
Flank/abdominal mass	4.4 (45/1,012)	0.4	1.8	0.0064
Hypertension	2.5 (25/1,013)	0.6	1.4	0.3945
Erythrocytosis	3.7 (28/755)	0.47	1.4	0.1810
Chills	3.1 (31/1,013)	0.6	1.7	0.0457
Cachexia related findings	35.3 (359/1,018)	0.4	3.5	<0.0001

Table I- 3: Presentation of localized/metastatic RCC and effect on disease free survival
The table shows the most common paraneoplastic syndromes and the percentage of occurrence. All paraneoplastic syndromes are more common in metastatic RCC. The significance was determined due to a multivariate analysis using each finding combined with T, M and N category ECOG-PS and Fuhrman grad. The definitions of hypoalbuminemia and weight loss are in the chapter above. Predictive for disease free survival were Hypoalbuminemia, Wt loss, Anorexia, Malaise (172)

5.2 Imaging investigation

Computerized tomography (CT) and magnetic resonance imaging (MRI) are used to characterize renal lesions and with these two imaging techniques, diagnosis of most renal masses is accurate. The most important criterion for differentiating malignant tumors is the presence of contrast enhancement. To show enhancement, imaging must be performed both before and after administering of intravenous contrast medium. In CT imaging, enhancement in renal masses is defined as change of 20 Hounsfield Unit (HU) readings before and after the contrast medium administered. (173, 174) MRI is indicated in some particular patients such as patients who react allergically to iodine containing contrast agents, pregnant patients and patients with impaired renal function. (175)
CT and MRI are not able to differ oncocytomas or a smooth muscle cell containing angiomyolipoma from malignant renal neoplasms. (176, 177)

Patients with any sign of impaired renal function should be offered an additional isotope renogram to optimize their treatment. For example, radical nephrectomy is a significant risk factor for the development of chronic kidney disease and might no longer be regarded as the gold standard treatment for small, renal cortical tumors. (178)

For the assessment of renal cystic masses, in 1986, Bosniak presented his classification, that classifies cystic renal lesions ranging from simple uncomplicated benign cysts to cystic malignancies. (179) The Bosniak classification suggests additionally the correct treatment for each category. Category I and II are classified as benign lesions. Category I is a simple benign cyst with a hairline thin wall. Category II is a benign cyst containing more hairline thin septa. This lesion has a sharp margin and no contrast enhancement. Category IIF should be followed up because a small proportion of these lesions have a malignant potential. These cysts may contain more hairline-thin septa in which enhancement may be seen. Category III contains over 50% of malignant lesions, therefore surgery or at least close follow up is highly recommended. These cysts are intermediate cystic masses that show thickened irregular walls and septa in which contrast enhancement occur. The last category is the Bosniak category IV. Bosniak IV cysts are mostly malignant and surgery is the treatment of choice. Category IV lesions are cystic tumors that contain enhancing soft tissue components. (180)

6. Prognostic Factors

In this chapter I will discuss the common prognostic factors for RCC such as anatomical factors, histological factors, clinical factors and molecular factors. I will also mention the most popular prognostic system like the stage, size, grad, necrosis-system (SSIGN) and the Memorial Sloan Kettering cancer center prognostic system (MSKCC).

6.1 Anatomical factors

Anatomical factors include tumor size, venous invasion, renal capsule invasion, adrenal invasion, lymph node and distant site metastasis. All of these factors commonly gathered together in the TNM classification system. (181)

The prognostic value of the 2010 TNM classification has been confirmed by an independent group. This study recommends the 2010 TNM classification system as a robust predictor for cancer specific survival. (182)

T - Primary tumour			
TX	Primary tumour cannot be assessed		
T0	No evidence of primary tumour		
T1	Tumour ≤ 7 cm in greatest dimension, limited to the kidney		
	T1a	Tumour ≤ 4 cm in greatest dimension, limited to the kidney	
	T1b	Tumour > 4 cm but ≤ 7 cm in greatest dimension	
T2	Tumour > 7 cm in greatest dimension, limited to the kidney		
	T2a	Tumour > 7 cm but ≤ 10 cm in greatest dimension	
	T2b	Tumours > 10 cm limited to the kidney	
T3	Tumour extends into major veins or directly invades adrenal gland or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota's fascia		
	T3a	Tumour grossly extends into the renal vein or its segmental (muscle-containing) branches or tumour invades perirenal and/or renal sinus (peripelvic) fat but not beyond Gerota's fascia	
	T3b	Tumour grossly extends into the vena cava below the diaphragm	
	T3c	Tumour grossly extends into vena cava above the diaphragm or invades the wall of the vena cava	
T4	Tumour invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)		
N - Regional lymph nodes			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in a single regional lymph node		
N2	Metastasis in more than 1 regional lymph node		
M - Distant metastasis			
M0	No distant metastasis		
M1	Distant metastasis		
TNM stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IV	T4	Any N	M0
	Any T	N2	M0
	Any T	Any N	M1

Table I- 4: 2010 TNM classification system

According to Sobin et al. (183)

Also the TNM stage grouping is a valid predictor for disease specific survival. (184)

6.2 Histological factors

Histological factors such as Fuhrman grade, histological subtypes of RCC, sarcomatoid transformation, microvascular invasion and tumor necrosis were used in the classification of RCC. (181)
Fuhrman grading is the most common histological grading system for RCC. (185) However, for chRCC, the CTG system is recommended. (151) Similar to other disciplines of medicine, diagnosis in histopathology depends on the observer. This problem is called inter-observer error. Additionally, the diagnosis differs of the same observer from one day to the other and this is called intra-observer error. Despite this intra-and inter-observer errors, Fuhrman grading has been shown to be an independent prognostic factor in RCC. (186)

The presence of a sarcomatoid component and nuclear grade were significantly associated with cancer- specific death from clear cell, papillary, and chromophobe RCC. Histologic tumor necrosis was significantly associated with cancer- specific death from clear cell and chromophobe RCC, but not with papillary RCC. In univariable analysis, there is a trend towards a better prognosis for patients with chRCC versus pRCC versus ccRCC. (187)

However, multivariable analyses demonstrated that TNM stage, Fuhrman grade and Eastern Cooperative Oncology Group Performance Status (ECOG PS), were retained as independent prognostic variables, but not histological subtypes. (188)

6.3 Molecular factors

Several molecular markers have been investigated. One study investigated osteopontin, carbonic anhydrase IX and C-reactive protein (CRP) as promising markers, but they did not improve the predictive accuracy of the current prognostic systems. (189)Another study tested the clinical effect of serum-VEGF and serum-fibronectin with no effect on predictive accuracy. (190)

To date, none of these markers has been shown to improve the predictive accuracy of current prognostic systems and their use is therefore not recommended in routine practice factors.

6.4 Prognostic systems

Postoperative prognostic systems and nomograms combine independent prognostic factors. There are a few different prognostic models both for localized and metastasized RCC. For the localized RCC are the University of California Los Angeles integrated staging system (UISS), the SSIGN and the post-operative Karakiewicz´s nomogram available. The most common prognostic models for metastasized RCC are the MSKCC- prognostic system and the Heng´s model. These models combine different variables and become more accurate than TNM system or Fuhrman nuclear grad alone. (191, 192, 193)
I only want to demonstrate one score for localized RCC and one for metastasized RCC. The other scores use different prognostic factors.

6.4.1 Stage, Size, Grade, Necrosis (SSIGN)

The stage, size, grade, and necrosis (SSIGN) score was developed by the Mayo Clinic to predict cancer-specific survival (CSS) of patients with ccRCC who underwent radical nephrectomy. The SSIGN score combines a few independent prognostic factors to a new predictive variable. This new variable allows clinicians predicting the prognosis of a patient with ccRCC with more accuracy than only with the single factors. In patients with ccRCC 1997 TNM stage, tumor size, nuclear grade and histological tumor necrosis were significantly associated with cancer specific survival. The SSIGN score is based on these features. T, N, M, tumor size, nuclear grade (Fuhrman) and the presence of necrosis get a number. If you add together the numbers of all categories you get a number between 0 and 15. The magnitude of this number behaves indirectly proportional to cancer specific survival. The aim of this prognostic system is to allow physicians to assess the effects of tumor characteristic outcome, which can be used to optimize patients management and treatment stratify patients for clinical trials and develop postoperative surveillance programs. (191) The SSIGN was externally validated by Zigeuner et al. who found the same results as the original study of the Mayo Clinic. This data support the routine use of the SSIGN score in clinical practice with regard to follow-up decisions and patient selection for adjuvant trials. (192)

Feature	Score
T stage:	
pT1	0
pT2	1
pT3a	2
pT3b	2
pT3c	2
pT4	0
N stage:	
pNx	0
pN0	0
pN1	2
pN2	2
M stage:	
pM0	0
pM1	4
Tumor size (cm.):	
Less than 5	0
5 or Greater	2
Nuclear grade:	
1	0
2	0
3	1
4	3
Necrosis:	
Absent	0
Present	2

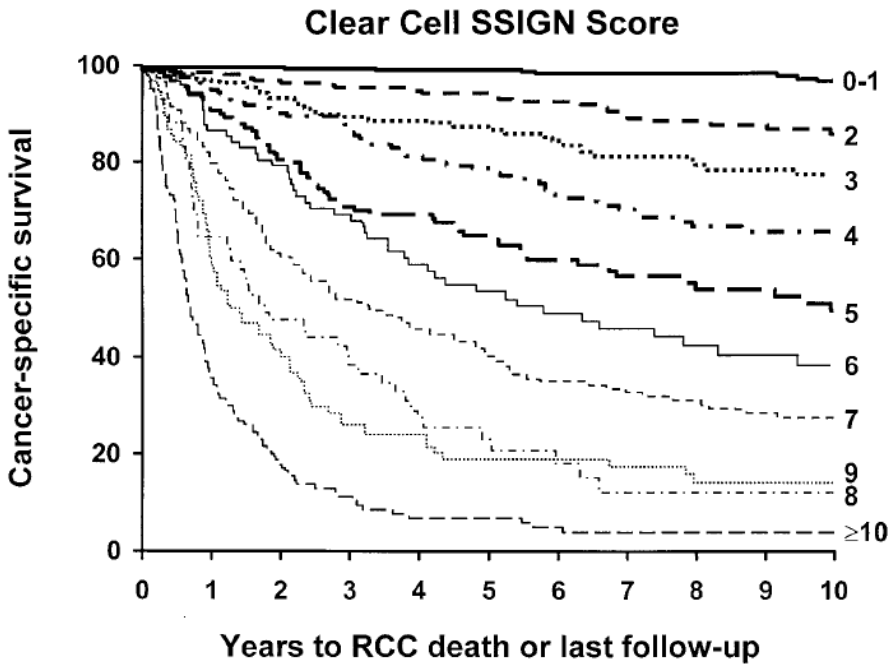

Table I- 5: SSIGN algorithm

The scores in the tables are added together and the total is used to determine survival using the Kaplan-Meier estimate on the right side. If you want to determine the exact cancer specific survival there is the possibility to get a result from another exact table instead of the Kaplan-Meier estimate (193)

6.4.2 Memorial Sloan Kettering Cancer Center (MSKCC) system

MSKCC prognostic system was introduced by Motzer et al. in 2002. MSKCC is prognostic model for metastasized RCC treated with IFN-α. Patients with a low risk (0 risk factors) show a mean survival of 30 months. Those patients with intermediate risk (1-2 risk factors) show a mean survival of 14 month and those with high risk (>3 risk factors) a mean survival of 5 months (194)

risk factor	limit
low Karnofsky-Index	<80%
high LDH	>1.5 times norm
low HB	< lower limit
high serum corrected Ca^{2+}	>10mg/dl
time from nephrectomy to metastasis	<1year

Table I- 6: risk factors to calculate the MSKCC score

7. Treatment of localized RCC

More than 50% of all RCCs diagnosed were found at localized stage (T1–T2N0M0 or stage I–II). Open radical nephrectomy has been the standard curative treatment for localized RCC in the past five decades. In last time nephron sparing surgery becomes the best treatment for localized RCC. (195)

7.1 Surgery versus non-surgical treatment

One matched pair study (derived from the SEER database) compared surgery for small renal masses (< 4cm) with non-surgical management. Included were pT1a patients who were assigned to either observation or active surveillance. The analysis showed that surgical therapy had a significant 5 year cancer-specific mortality benefit over non-surgical intervention. However, even though this study was matched, it is marked by allocation bias; the patients assigned to the surveillance arm were older, more frail, and less suitable candidates for surgery. There was no comparative study addressing this comparison in terms of perioperative and QoL outcomes (196,197)

7.2 Radical nephrectomy versus partial nephrectomy

There have been numerous studies comparing partial (nephron sparing surgery) and radical nephrectomy. The cancer-specific 5-year survival rates did not differ between the radical and partial nephrectomy group. However, the mean postoperative serum creatinine levels were significantly higher than the mean preoperative levels for patients in the radical nephrectomy group. (198, 199) Radical nephrectomy causes a major impairment of renal function compared with the partial nephrectomy. Moreover, patients undergoing RN have a greater chance of developing renal failure and higher mortality rates. For small RCCs partial nephrectomy should, whenever possible, be regarded as the primary therapeutic option, given that it obtains similar oncological outcomes to radical nephrectomy and preserves renal function, which seems to translate into a lower overall mortality rate. (200) Up to this point we know that partial nephrectomy is recommended as treatment of small renal masses. The limits of partial nephrectomy are discussed controversially. Patard et al. published 2004 that patients with T1b (4-7cm size) tumors show no significant difference in the rate of cancer specific deaths between patients undergoing partial and patients

undergoing radical nephrectomy. Regarding this study it is safe to treat T1N0M0 tumors up to 7 cm with partial nephrectomy when technically feasible. (203)

7.3 Techniques of radical and partial nephrectomy

7.3.1 Techniques of radical nephrectomy

Studies found similar oncological outcomes for laparoscopic vs. open radical nephrectomy. Data from one RCT (201) and two NRSs (202) showed a significantly shorter hospital stay and lower analgesic requirement for the laparoscopic radical nephrectomy group compared with the open group.

Convalescence time was also significantly shorter (202). There was no difference in the number of patients receiving blood transfusions between the two surgical approaches, but the peri-operative blood loss was significantly less in the laparoscopic arm in all three studies (204). Surgical complications were marked by low event rates and very wide confidence intervals. There was no difference in complications but the operation time was significantly shorter in the open nephrectomy arm. The post-operative QoL scores were similar between the two groups (204).

In regard to the best approach for performing radical nephrectomy, both retroperitoneal or transperitoneal approaches had similar oncological outcomes in the two RTCs (206)

7.3.2 Techniques of partial nephrectomy

Studies comparing laparoscopic partial nephrectomy and open partial nephrectomy found no difference in PFS and OS between the two techniques in centres with laparoscopic expertise. The mean estimated blood loss was generally found to be lower with the laparoscopic approach. More blood transfusion events occurred in the laparoscopic group. No significant differences were found between the two approaches in post-operative mortality events. However, the operative time was generally significantly longer in the laparoscopic group. The warm ischaemia time was found to be shorter with the open approach (207)

At present, no study has compared the oncological outcomes of robot-assisted vs. laparoscopic partial nephrectomy. A prospective comparison of surgical outcomes obtained after robotic or pure laparoscopic partial nephrectomy in moderate-to-complex renal tumors showed a significantly lower estimated blood loss

and a shorter warm ischaemia time in the robotic group (287). Two recent meta-analyses of relatively small series showed comparable peri-operative outcomes and a shorter warm ischaemia time for robot-assisted partial nephrectomy (288).

7.4 Adrenalectomy

Adrenalectomy should not be routinely performed during partial or radical nephrectomy, even for upper pole tumors. Adrenalectomy is only recommended if a suspicious adrenal lesion is identified radiographically or invasion of the adrenal gland is suspected intra-operatively. (195, 205)

8. Treatment of metastasized RCC

8.1 Local treatment of metastatic renal cell carcinoma

Metastasized RCC has very poor prognosis and outcomes with a median survival of approximately 7 months had been reported in historical series. It has been suggested, that cytoreductive nephrectomy is able to improve overall survival. (208) In 2004 Flanigan et al. published a meta-analysis of 2 prospective studies regarding this issue. (208)They found a significant increase of overall survival in the cytoreductive nephrectomy + IFN-α group compared with the IFN-α only group. The results were 13.6 months (cytoreductive +IFN-α) versus 7.8months (only IFN-α). These results represent a 31% decreased risk of death in the cytoreductive nephrectomy + IFN-α group in the first year after diagnosis. Regarding surgery associated complications in patients with a good performance status, this meta-analysis showed that only 5.6% had suffered from grade IV complications and even more, 76, 6% had no complications after surgery. (208)
Another retrospective study compared cytoreductive nephrectomy + VEGF targeting therapies versus the treatment with VEGF targeting drugs only. (209)This study also shows an increased median overall survival for the cytoreductive nephrectomy + VEGF targeting drug group. Even patients with a Karnofsky-Index <80% (= ECOG-PS 1) seem to benefit from cytoreductive nephrectomy. (209)Beside these positive effects on survival of patients with metastasized RCC, cytoreductive nephrectomy is a possible palliative intervention to control local symptoms such as gross haematuria and flank pain. (2)

Other problems in metastasized disease are the affections caused by the tumor resettlement in different organ systems. It has been shown, that the complete resection of metastases called complete metastasectomy is able to increase the overall survival. (210) Different organ localizations of metastases have been investigated. Regarding bone metastases, surgical treatment is able to improve the survival rates. However, it is not necessary to perform wide resections and reconstructions of the bone metastases, because there seems to be no advantage compared to intra-lesional resection. (211) As an alternative to surgical treatment of bone metastases, external radiation is used as treatment choice for local tumor control, especially for pain reduction. (212) With regard to visceral metastases such as liver, pancreas and lung metastases, patients also seem to benefit from metastasectomy. They show a significant increased overall survival compared to non-surgical treated patients. (213, 214, 215)

8.2 Systemic therapy for metastatic renal cell carcinoma

This chapter is structured chronologically. I will start with the oldest therapy the cytotoxic-chemotherapy which was not very effective and had a lot of complications. The first real breakthrough in systemic therapy of metastasized RCC was the immunotherapy (interferon-α and interleukin-2) which were established in the 1990. Finally I will fall in with the new VEGF and mammalian target of rapamycin (mTOR) targeting drugs which are nowadays the recommended treatment for metastatic RCC. I will also present a promising new way to treat end-stage metastasized RCC which doesn't respond to any other agent.

8.2.1 Chemotherapy

As most RCC develop from the proximal tubules, they have high levels of multidrug resistance proteins such as P-glycoprotein, the prototype of MDR-proteins, and therefore are resistant to most chemotherapies. (216) P-glycoprotein over-expression protects kidney cancer cells and normal kidney tubules from penetration by drugs and toxins. (217) Beside the P-glycoprotein detoxifying enzymes such as glutathione-S-transferase may contribute to the chemoresistance of RCC. (218) A variety of chemo- and combined chemoimmunetherapies have been investigated. In the 1990s, floxuridine and fluorouracil (5-FU) have been extensively investigated as promising agents in metastatic RCC, because

they are not removed from the tumor cells by any MDR-like mechanism. But all hopes that were set in this drug were demolished by Yagoda et al. who reported overall response rates of only 6% and the duration of the response was only a few months. (219) Another cytotoxic agent of this era was vinblastine, a vinca alkaloid which inhibits the growth of the microtubules was also judged as ineffective with response rates of only 3%. (220) To date, no effective mono therapy with a cytotoxic chemotherapy has been described.

In the 2000s, a variety of combination therapies had been studied. The FOLFOX regime, a chemo regime effectively used in colorectal cancer, was tested and failed in metastatic RCC. These results pointed out, that oxalipatin plays no role in the treatment of metastasized RCC. (221) Gemcitabine is an agent, which is no substrate of P-glycoprotein and thought to be a novel promising drug in the high level P-glycoprotein containing RCC. Gemcitabine leads to moderate responses, either alone, or in combination with 5-FU, but no further improvements were recorded when cisplatinum, interferon, or interleukin-2 were added. (222) Ryan et al. investigated the utility of combinations of gemcitabine with 5-FU and antimetabolite and Gemcitabine + 5-FU + immunotherapy and found response rates of 14 and 17%, respectively. (223) Another area of investigation were alkylating agents. Within alkylating chemo agents, temozolomide exhibited a broad antitumor activity in patients with metastatic RCC in a phase I trial. Park et al. verified these results in a phase II trial, but the final results were disappointing. (224)

A breakthrough in the treatment of metastatic RCC was made, when IFN-α and IL-2 showed sustained activity against this disease. At first, these immune therapies were combined with conventional chemotherapies. In a prospective randomized study, IFN-α showed equivalent efficiency to combination of IFN-α + IL-2 + 5-FU. (225)

	Antimetabolites	5-FU, floxuridine; gemcitabine; capacitabine; troxacitabine	No. patients	Overall response %
Monotherapy		Oevermann et al. (2000)	30	10
		Fizazi et al. (2003)	42	2
		Townsley et al. (2003)	33	2
	Platina compounds	Cisplatinum; oxaliplatin		
		Chaouche (2000)	16	0
		Rini et al. (2000)	39	17
	Alkylating compounds			
		Park et al. (2002)	12	0
	Irofulven			
		Berg et al. (2001)	12	0
		Amato RJ (2002)	20	0
Combinations	Cytokines with antimetabolites	IFN; IL-2; 5-FU		
		Rini at al. (2000)	39	7
		Bennouna et al. (2003)	59	0
		Haas et al. (2003)	21	1
		Porta et al. (2004)	42	11
		Atzpodien et al. (2004)	132	31
	Cytokines with antimetabolites and alkylated compounds or taxanes	IFN;IL-2; CRA; paclitaxel; vinblastine; 5-FU;temozolomide		
		Fosså et al. (2004)	53	19
		Atzpodien et al. (2004)	146	26
		Sunkara et al. (2004)		
		Bacoyiannis et al. (2002)	40	8
		Negrier et al. (2000)	131	0
		Vaishampayan et al. (2001)	20	5
		Gez et al. (2002)	62	29

Table I- 7: Monotherapies and combination therapies with cytotoxic agents
The table shows a few chemotherapies which were considered in treatment of metastasized RC. The overall response rates and the number of patients included were low and the results are unsuccessful. (226)

8.2.1.1 Hormone therapy

The discovery of estrogen receptors in metastatic RCC encouraged the use of hormones in animal studies. The gestational agent medroxyprogesterone and antiestrogens such as tamoxifen have been verified in several trials. Despite all promises, the clinical response rates have been rather low. (227)

8.2.2 Immunotherapy

Interferon-alpha has an overall response rate of 10-15% in phase II and III clinical trials and was considered a standard option for patients with metastasized RCC in the pre-targeted therapy era. One trial tried to show an advantage of a combination with toremifen a selective estrogen receptor modulator but failed in this effort. (228)

Immunotherapy is based on the fact, that in patients with mRCC, spontaneous regression of metastases after a cytoreductive nephrectomy could occur. According to many reviews describing spontaneous regression, RCC is one of the most frequent types of malignancies related to this phenomenon. The frequency of spontaneous regression in RCC patients is estimated to be about 1%. Spontaneous regression is defined as partial or complete disappearance of a tumor without any treatment or with treatment which is generally

perceived as irrelevant to the progression of cancer, but this definition does not mean the cure of cancer.

In the last 5-years, new molecular targeted drugs have changed pharmacotherapy of metastasized RCC. Previously, cytokine therapy, consisting of interleukin-2 and interferon-α, was used. IL-2 and IFN-α were associated with low rates of responses, high toxicity and side effects. However, the combination of those cytokines with new targeting therapies is still in use. The natural glycoprotein interferon-alpha (IFN-α) stimulates host mononuclear cells and is clearly involved in host immune surveillance. IFN-α induces expression of major histocompatibility complex molecules and tumor-associated antigens as well as enhancing the function of T-cell and dendritic cells (DC). Dendritic cells belong to antigen-presenting cells, which are able to activate an inflammatory anti-tumor reaction.IL-2 stimulates natural killer cells and helper T-cells in order to destroy the tumor; however IL-2 has no direct effect on the tumor. The role of the relative increase in the number of elements of the immune system is more valuable than the absolute increase, caused especially by CD8+ cells. (229)

Beside IL-2 and IFN-α, immunotherapy includes vaccination therapy. A trial, performed by Schwab, suggested, that the interaction between DC vaccination and cytokine therapy is able to optimize the clinical benefit in metastatic RCC. (230)

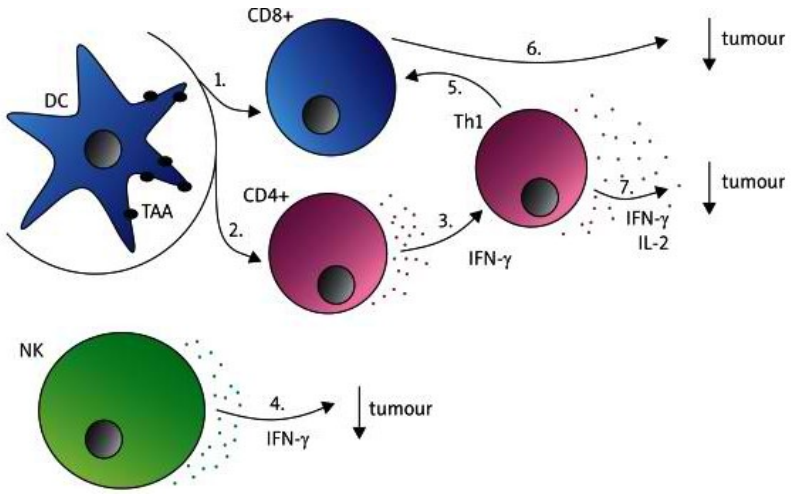

Figure I -9: The role of tumor response in tumor rejection

Dendritic cells (DC) presenting tumor associated antigens (TAA) migrate to the lymph nodes where they interact with T-lymphocytes, activating CD8+ **(1)** and CD4+ **(2)** cells. Proliferating CD4+ cells develop into effector T-cells – T-helper cell type 1 (TH1) **(3)**. Natural killer cells secreting IFN-γ activate cytolytic effector cells, which promote the expression of Fas-ligand, which induces apoptosis of tumor cells **(4)**. TH1 activating CD8+ **(5)**.CD8+ cells destroys tumor cells **(6)**. TH1 releases IFN-γ and IL-2 which mobilizes the immune effector cells **(7)**. (229)

A large Cochrane Meta-Analysis summarized 53 studies. Combined data for a variety of immunotherapies gave an overall chance of partial or complete remission of only 12.9% compared to 2.5% in non-immunotherapy control arm, and 4.3% in placebo arm. 28% of these remissions were evaluated as complete remission and the median survival was averaged 13.3 months. (231)

8.2.2.1 Interferon–α (IFN-α)

Patients with no or less primary therapy, stratified at good risk according to the MSKCC (Motzer et al.) criteria, only lung metastases and clear cell histology show the best responses to IFN-α. IFN-α provided a response rate of 6-15%, together with a 25% decrease in the risk for tumor progression and a modest survival benefit of 3-5months in comparison with placebo.(231, 233) Most trials evaluated the benefit of IFN-α in the MSKCC- good prognosis group. But, do cytokines improve survival in patients with intermediate prognosis? For this purpose, untreated patients with more than one metastatic site and Karnofsky score 80 were randomized to medroxyprogesterone, -Interferon (IFN), subcutaneous IL2 or IFN+ subcutaneous IL2 in a 2x2 factorial design. Response rates and overall survival were very low in this trial. The intermediate risk group seems to be the reason for this poor prognosis. (233)

Although IFN-α associated toxicity is not life threatening, it shows severe side effects. The main side effects of IFN-α therapy are represented by flu-like symptoms such as weakness, fatigue, myalgia, fever and anorexia. In most cases, the symptoms subsided with duration of IFN therapy. However, when the toxicity became too severe, therapy must be interrupted or the intensity had to be yielded. (231)

8.2.2.2 Interleukin-2 (IL-2)

The immune stimulatory cytokine interleukin-2 (IL-2) was first given, along with lymphokine activated killer cells (LAK), to RCC patients with advanced cancer in the early 1980s. (234) IL-2 has curative potential in a small subpopulation of patients with metastatic RCC. The complete response rate to high-dose intravenous IL-2 is between 6.6% and 9.3% in patients with metastasized melanoma and clear cell RCC, and the majority of these completely responding patients will not relapse according to follow-up data extending to 17 years. (235) Other trials could show response rates including complete and partial responses between 7-27%. (236, 237) One trial compared the effectiveness of IL-2 versus IL-2plus IFN-α. The response rate was 23.2% for high dose IL-2 versus 9.9% for IL-2/IFN-α. The progression free survival seems to be better in the high dose IL-2 group but these results were not significant. For patients with bone or liver metastases or a primary tumor in place, survival was significantly superior with high dose IL-2 compared with the combination IL-2/IFN-α. This trial supported high dose IL-2 in the treatment of selected patients with metastasized RCC. (238)IL-2 shows in comparison to IFN-α significant multisystem toxicity resulting in treatment-related mortalities of 1% to 4%. (239). This led to experimentation with lower dose regimens of interleukin therapy. The optimal IL-2 regime is not clear, but one trial compared low dose versus high dose IL-2 and found a clear advantage for high dose bolus IL-2. This trial demonstrated a significantly higher response rate with high dose intravenous IL-2 (21%) versus low dose intravenous IL-2 (13%) but no overall survival difference. Response durability and survival in completely responding patients were also superior with high dose intravenous IL-2 compared with low dose IL-2 therapy. However, low dose shows less toxicities. IL-2 related death did not occur in both high and low dose IL-2. (240)
The big disadvantages of IL-2 therapy are, that only clear cell-type RCC seems to respond to immunotherapy and to date, IL-2 has not been validated in comparison with best supportive care. (231)

	High–Dose	Low–Dose	Subcutaneous
Total courses (100%)	285	272	181
Thrombocytopenia	9.2	1.5	0
Hyperbilirubinemia	3.2	0.7	0
ALT	3.2	0.7	0.6
Nausea/vomiting	13.4	8.5	3.3
Diarrhea	9.2	3.7	1.7
Peripheral edema	0.4	2.6	0
Creatinine (≥ 8.0)	1.1	2.6	0.6
Oliguria (≤ 80 mL/8 h)	12.0	7.7	1.1
Pulmonary	4.2	1.1	0
Malaise	20.5	9.9	9.4
Infection	2.8	2.6	1.1
Arrhythmia, atrial	4.2	1.5	0
Hypotension	36.4	2.9	0
CNS level of consciousness	2.5	2.6	0
CNS orientation	10.2	3.7	1.7
Death	0	0	0

Table I- 8: Toxic effects and the distribution of abundances IL-2 therapy
The table compares the toxicity of 720,000 U/kg (high-dose) IL-2 or 72,000 U/kg (low-dose) IL-2, both given by intravenous (IV) bolus every 8 hours and low-dose daily subcutaneous IL-2. (240)

8.2.2.3 Vaccination therapy

It is well known, that RCC represents one of the most immune-responsive tumors. Cancer vaccines offer a possible approach to achieve better response and overall survival rates in metastatic RCC. To date, only a few potentially interesting tumor-associated antigens (TAA) have been identified in RCC. Cancer vaccines could be divided into three groups: autologous tumor cell vaccines, gene-modified tumor vaccines, and dendritic cell (DC)–based vaccines. A lot of vaccines had been tested in clinical trials, but none of these vaccines both as monotherapy and combined with cytokines was able to improve the clinical outcome in patients with metastatic RCC. (241,242)
However, one study seems to be promising. This phase I trial investigated the clinical utility of vascular endothelial growth factor receptor 1 (VEGFR1) peptide vaccination. The results of this trial shows that VEGFR1 peptide vaccine is safe in usage (no patients showed any toxicity greater grade 3) and partial responses plus a stable disease for 5 month. Regarding these results VEGFR1peptide vaccine could be recommend for further trials. (243)

8.2.2.4 Targeted immunotherapy

The programmed death 1 (PD-1) protein, a T-cell co inhibitory receptor, and one of its ligands, PD-L1, play a pivotal role in the ability of tumor cells to evade the host's immune system. Blockade of interactions between PD-1 and PD-L1 enhances immune function in vitro and mediates antitumor activity in preclinical models. The infusion of PD-L1 antibodies (10mg/kg in a 6 week cycle as a 60 minutes intravenous infusion at days 1, 15, 29) had been revealed as an effective therapy in metastasized RCC and showed an objective response rate of 12% with responses lasting from 4 to 17 months. Fourty-one percent of patients with metastasized RCC showed a stable disease lasting at least 24 months. Most patients were pretreated with nephrectomy (94%), anti-angiogenic drugs (82%), and immune therapy (41%). (244) Targeted immunotherapy with anti – PD-L1 antibodies seems to be a promising approach as second line therapy after prior tyrosinekinase inhibitor therapy. Immune checkpoint inhibitors like PD-L1 (especially nivolumab) may play a key role in the future of management of solid tumors including kidney cancer. (245)

8.2.3 Drugs targeting VEGF, other receptor kinases and mTOR

Recent advances in molecular biology and molecular chemistry led to the development of several novel drugs to treat metastatic RCC.

Vascular endothelial growth factor (VEGF) and its receptor play a fundamental role in tumor angiogenesis. The loss of the short arm of chromosome 3 and thus loss of VHL-tumor suppressor gene lead in VHL-syndrome and in most sporadic forms of RCC to a hypoxia inducible factor –α (HIF-α) dysregulation. This dysregulation leads to increased expression of VEGF and other proangiogenic factors which supports stability, proliferation, migration, and survival, contributing also to tumor cell metabolism and drug and radiation resistance. (246) Beside VEGF- pathway several other signaling pathways such as PDGF – mammalian target of Rapamycin are involved in tumor angiogenesis and play a crucial roles in RCC carcinogens. (247)

To date, several drugs have been approved in the clinical treatment of metastasized RCC: Sorafenib (Nexavar®), Sunitinib (Sutent ®), Bevacizumab (Avastin ®), Pazopanib (Votrient ®), Temsirolimus (Torisel ®), Everolimus (Afinitor ®), Axitinib (Inlyta ®)

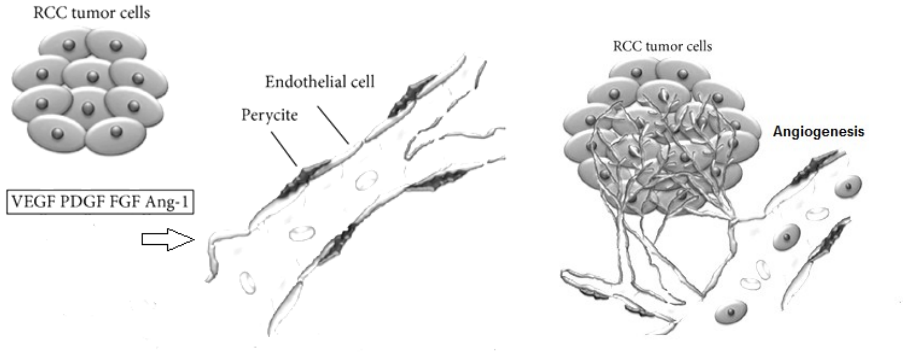

Figure I -10: RCC and tumor angiogenesis

(A) Factors influencing promotion of tumor angiogenesis. **(B)** Tumor vessel sprouting and metastasis. Ang-1; angiopoietin-1; FGF; fibroblast growth factor; PDGF; platelet derived growth factor; RCC; renal cell carcinoma; VEGF; vascular endothelial growth factor. (247)

8.2.3.1 Summary of Pharmakodynamics of targeting drugs

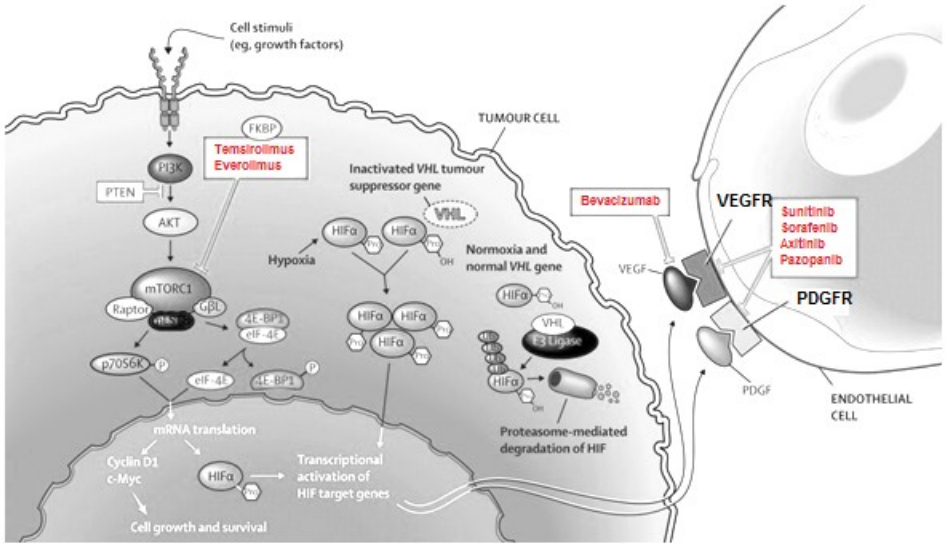

Figure I -11: Biological pathways and therapeutic targeted agents for RCC

During normoxia, HIFα is hydroxylated at one of two proline residues via an oxygen-dependent enzymatic mechanism. The VHL complex binds to the hydroxylated HIF-1α and polyubiquinates HIF-1α, leading to proteosome-mediated degradation of HIF-1α. In the absence of VHL (or during hypoxic conditions), HIFα accumulates and binds with its constitutively present partner HIFβ. The HIF complex translocates to the nucleus and binds to HIF-responsive element (HRE) enhancer sequence, leading to transcription of hypoxia-induced genes, including VEGF-A and PDGF. These growth factors are secreted into the

extracellular space and can either via paracrine action, bind to RTKs located on stromal or endothelial cells, leading to stromal proliferation and angiogenesis or via autocrine action, bind to RTKs located on tumor cells, leading to proliferation and survival. For example, RTKs can stimulate the mitogenic RAS/Raf/MEK pathway, as the phosphotyrosines of RTKs facilitate docking of Grb2-SOS complex, ultimately resulting in activation of Ras. The activated Ras binds to Raf-1; afterwards, Raf-1 is activated via a complex series of phosphorylation and dephosphorylation steps. Similarly, mTOR is stimulated by a phosphorylation cascade, which involves proteins, including phosphatidylinositol 3-kinase (PI3K) and AKT. Once stimulated, mTOR controls protein translation of elements involved in cell cycle progression; in addition, mTOR also controls protein synthesis of HIF-1α in RCC cells. The signal pathways in RCC can be inhibited at several steps, including the following: **(A)** inhibition of VEGF (by bevacizumab); **(B)** inhibition of tyrosine kinase activity of RTK (by sunitinib, sorafenib, axitinib, pazopanib); and **(C)** inhibition of mTOR (by temsirolimus and everolimus). (170, 248, 249) **Abbrevations:** FKBP=FK binding protein; PI3K=phosphoinositide 3-kinase; AKT=protein kinase B; mTORC1/2=mammalian target of rapamycin complex 1/2; mLST8=mammalian lethal with SEC13 protein 8; 4E-BP1=4E binding protein-1; eIF-4E=eukaryotic initiation factor-4 subunit E; VEGFR=vascular endothelial growth factor receptor; PDGFR=platelet-derived growth factor receptor; Pro=proline; P=phosphorous; Ub=Ubiquitin; FGF=fibroblast growth factor; IL-8=interleukin-8; PlGF=platelet-derived growth factor; MSIN1=mammalian stress–activated protein kinase–interacting protein 1; RTK=receptor tyrosine kinases

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8.2.3.2 Tyrosine kinase inhibitors

Sorafenib (Nexavar®)

Sorafenib is an oral multikinase inhibitor with activity against Raf-1 serin/threonin kinase, B-Raf, vascular endothelial growth factor receptor -2 (VEGFR-2), platelet derived growth factor receptor (PDGFR), FMS-like tyrosine kinase 3 (FLT-3) and c-KIT. Escudier et al. compared Sorafenib to placebo in a randomized, double-blind trial. They found an improvement in progression free survival of 3 months in favor of sorafenib in patients after failure of previous cytokine therapies (250)

Sunitinib (Sutent®)

Sunitinib is an oxindol tyrosine kinase inhibitor. It selectively inhibits PDGFR, VEGFR, c-KIT and FLT-3 and has antitumor and anti angiogeneic activity. Sunitinib achieved an improvement both on progression free survival and overall survival compared with IFN-α as monotherapy in metastasized RCC. Progression free survival was 11 months in the Sunitinib group versus 5 months in IFN-α group, the benefit in favor of sunitinib was confirmed in overall survival (26.4 months versus 21.8 months). IFN-α was clearly inferior to Sunitinib in MSKCC low risk and intermediate risk patients. (251) Moreover, the specific dose regimen of Sunitinib was investigated. Therefore, a randomized trial with sunitinib on an intermittent (50 mg/day 4 weeks on/ 2weeks of) versus continuous uninterrupted dosing (37, 5 mg/day) schedule as first-line therapy in metastasized RCC had been performed. In both groups, there was no significant difference found regarding adverse events, overall survival and the progression free survival. Because of the statistically non-significant but numerically longer time to progression compared to the standard continuous therapy with a dosage of 50 mg/day, the authors recommend maintaining the standard therapy. (252)

Pazopanib (Votrient®)

Pazopanib is an oral angiogenesis inhibitor that targets VEGFR, PDGFR and c-KIT.
In a randomized, double-blinded placebo controlled phase III trial, pazopanib was superior to placebo. Pazopanib significantly prolonged progression free survival compared to placebo. The objective response rate was 30% with pazopanib, compared with 3% with placebo, respectively. The most common adverse events were diarrhea, hypertension, hair color changes, nausea, anorexia, and vomiting and there was no evidence of clinically important differences in quality of life for pazopanib versus placebo.
These results established Pazopanib as first line option in the treatment of metastatic RCC. (253) Pazopanib and Sunitinib provided a progression-free survival benefit, as compared with placebo or interferon. The recently published COMPARZ trial investigated this issue. Pazopanib was non inferior to sunitinib with respect to progression-free survival. (HR for progression of disease or death from any cause was 1.05 [0.90 to 1.22]) Overall survival was similar in the Pazopanib and Sunitinib group. Patients treated with sunitinib, as compared with those treated with pazopanib, had a higher incidence of fatigue (63% vs. 55%), hand-foot syndrome (50% vs. 29%), and thrombocytopenia (78% vs. 41%); patients treated with pazopanib had a higher incidence of increased levels of alanine

aminotransferase (60%, vs. 43% with sunitinib). Pazopanib and Sunitinib show the same efficiency but safety and quality-of-life profiles favor pazopanib as first line option. (254)

Axitinib (Inlyta®)

Axitinib is an oral selective second generation inhibitor of VEGFR-1, -2 and -3 that blocks VEGFR receptors at subnanomolar drug concentrations with minimal inhibition of other targets. The trial which compared axitinib with sorafenib in patients, in whom previous cytokine therapy or targeted agents had failed, is called AXIS trial. The AXIS trial demonstrated a significantly longer progression free survival for axitinib compared with sorafenib. The most common adverse events under Axitinib therapy were diarrhoea, hypertension, and fatigue. Axitinib is a treatment option for second-line therapy of advanced renal cell carcinoma. (255)

Tivozanib (AV-951)

Tivozanib is also an oral selective tyrosine kinase inhibitor of all three VEGFR subtypes. Tivozanib demonstrated improved progression-free survival (PFS), but not overall survival (OS), and a differentiated safety profile, compared with sorafenib, as initial targeted therapy for metastatic RCC. Tivozanib showed an improved PFS (11.9months versus 9.1months). However, OS analyses showed a trend towards the sorafenib group, but these results were not significant. Regarding adverse events, hypotension (44% versus 34%) and dysphonia (21% versus 5%) were more common in the Tivozanib group but hand-foot skin reaction (54% versus 14%) and diarrhea (33% versus 23%) were more common in sorafenib group. (256) Due to the lack of an overall survival advantage, Tivozanib was not registered by the “Food and Drug Administration” (FDA)

8.2.3.3 Monoclonal antibody against circulating VEGF

Bevacizumab (Avastin®) is a humanized monoclonal antibody that binds VEGF subtype A, a subtype of VEGF. Bevacizumab was associated with an increase in OS and PFS in patients which were refractory to immunotherapy compared with placebo. (257) The best effects with Bevacizumab were achieved when combined with IFN-α.. The AVOREN trial confirmed these thoughts. Overall response was significantly higher in the Bevacizumab + IFN-α compared with IFN-α (31% response versus 13% responses in the only IFN-α arm).

Also PFS and OS increase under combined therapy. However, these benefits were only seen in intermediate and low risk patients. High risk patients have no benefit from this combination. (258)

8.2.3.4 Mammalian target of rapamycin (mTOR) inhibitors

Everolimus (Afinitor®)

Everolimus is a derivate of sirolimus (= rapamycin) and works similar to sirolimus as an inhibitor of mTOR. It is currently used as an immunosuppressant and treatment of renal cell cancer and other tumors. (259) A phase III study compared Everolimus + best supportive care with placebo + best supportive care and found a significantly prolonged progression free survival (4 months in Everolimus + BSC versus 1.9 months placebo + BSC) in patients in whom a previous therapy with TKI failed. According these findings Everolimus could be recommended as second line therapy after failure of prior TKI therapy. (260)

Temsirolimus (Torisel®)

Temsirolimus is a specific inhibitor of mTOR. Treatment with Temsirolimus leads to cell cycle arrest in the G1 phase, and also inhibits tumor angiogenesis by reducing synthesis of VEGF. (261) Temsirolimus was tested in the poor risk group against IFN-α and could assert. The results were significant in favor of Temsirolimus when it's compared with IFN-α as first line therapy in the MSKCC – poor risk group (OS was 10. 9 months versus 7. 3 months). However, a combination of both agents was not able to improve any clinical parameter. (262) Summarized, Temsirolimus could be recommended as first line option for poor risk group as only agent.

8.3 Recommendation of the EAU

RCC type	MSKCC risk group (3)	1st-line therapy*	2nd-line therapy*†	3rd-line therapy
Clear cell	Favourable or intermediate	• Sunitinib [1b] • IFN-α + bevacizumab [1b] Pazopanib ‡ [1b] *In selected patients:* • IFN-α [1b] • High-dose IL-2 [1b]	*After prior TKI:* • Axitinib [1b] • Sorafenib [1b] • Everolimus [1b] *After prior cytokines:* • Sorafenib [1b] • Axitinib [1b] • Pazopanib [1b]	• Everolimus after prior TKI(s) [1b]
	Poor ¶	• Temsirolimus [1b]		
Non-clear cell	Favourable	§		
	Intermediate	§		
	Poor	§		

Table I- 9: EAU 2013 evidence based guidelines

* Doses: IFN-α9 MU three times per week subcutaneously, bevacizumab 10 mg/kg biweekly intravenously; sunitinib 50 mg daily orally for a period of 4 weeks, followed by 2 weeks of rest (37.5 mg continuous dosing did not show significant differences); temsirolimus 25 mg weekly intravenously; pazopanib 800 mg daily orally. Axitinib 5 mg twice daily, to be increased to 7 mg twice daily, unless greater than grade 2 toxicity, blood pressure higher than 150/90 mmHg, or the patient is receiving antihypertensive medication.

† Listed in the order of data quality.

‡ Initial phase III study; involved a substantially smaller number of patients than in phase III studies of other targeted agents.

§ No standard treatment available. Patients should be treated in the framework of clinical trials. If a trial is not available, adecision can be made in consultation with the patient to perform treatment in line with clear cell renal cell carcinoma.

¶ Poor risk criteria in the NCT00065468 trial consisted of MSKCC risk plus metastases in multiple organs.Accordig to Ljungberg et al. (197)

II. Chapter: Utility of frozen section analysis in RCC treatment

1. Introduction

One of the basic principles of partial nephrectomy is resection of the tumor with normal tissue margins verified by frozen section analysis. In case of positive tumor margins, the surgeon is committed to complete the local resection in sano or to perform radical nephrectomy.
(264,265)

1.1 Frozen section procedure

The frozen section procedure (FS) is a pathological laboratory procedure which is used for microscopic investigation of tissue specimens. FS procedure is usually performed during surgery and influences the further proceeding of surgery. The aim of FS is the quick histological diagnosis of a macroscopic uncertain lesion. The performance of this method is appreciable and plays an important role in a lot of surgical procedures.
The most common questions of the surgeon to the frozen section are:

- Diagnosis of tissue type (e.g.: tumor or non neoplastic tissue)
- Diagnosis of tumor entity(benign versus malignant)
- Diagnosis of positive surgical margins (263)

1.1.1 Technique of frozen section

First step in the FS-diagnosis process is the macroscopic evaluation of the tissue specimen by the pathologist. The key instrument for FS is the cryostat, which is essentially a microtome inside a freezer (temperature about –20 to –30 °C). First the surgical specimen is embedded in a gel like medium consisting of poly ethylene glycol and polyvinyl alcohol and then rapidly frozen. Subsequently it is cut frozen with the microtome portion of the cryostat to a sections as thin as 1 µm then the section is picked up on a glass slide and stained with hematoxylin and eosin and viewed under the microscope.
FS is much more rapid than conventional preparation of histological samples. However, the quality of the sample is much lower. (263)

2. Evidence acquisition

Data for Chapter II – Utility of frozen section analysis in RCC were acquired by searches of PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using combinations of these terms: frozen section, renal cell carcinoma, cystic renal cell carcinoma, oncocytoma, utility, partial nephrectomy, nephron sparing and surgical margins. References from the identified articles were also investigated. Only papers published in English with no date restrictions and human studies were included.

3. Evidence synthesis

3.1 Frozen section and diagnosis of positive surgical margins

Historically, the reference standard for treatment of renal cell carcinoma has been radical nephrectomy as described by Robson (264) However, during past decades due to the development of modern imaging techniques kidney tumors are detected at earlier stages and partial nephrectomy has gained acceptance in the treatment of small renal masses. Partial nephrectomy has been shown to provide cancer free survival comparable to radical nephrectomy and the advantage of a reduced risk of cardiovascular events that directly translates into improved overall survival. (198, 199) The main concern of partial nephrectomy is the risk of local tumor recurrence, because of incomplete tumor removal.

Does surgical approach influence PSM?

However, a positive surgical margin (PSM) in partial nephrectomy (PN) is a rare event. A series of trials investigated the influence of the surgical technique on the occurrence of PSM. According to surgical technique, PSM rate after open partial are between 0% (265) and 5 % (266), PSM rates after laparoscopic PN differ between 0.7 and 4% (270) and one study demonstrated PSM rates ranging from 3.9 (268) to 5.7% (269) during robot-assisted partial nephrectomy. One study clearly showed that the PSM are much higher in patients in which PN is performed under imperative indication such as solitary kidney. In these patients PSM were observed in 15% of cases. (270)

Does the tumor size influence PSM?

Another factor that seems to influence PSM rates is the tumor size. This subject is still under debate because the studies which were performed regarding this issue are controversial. Patard et al. found comparable PSM rates in tumors smaller and larger than 4cm. (271), but higher PSM rates with decreasing tumor size were also observed. (272) Small tumor size might prevent the surgeon from accurately estimating tumor extension and thereby provoke a PSM. (267)

Does the histological subtype influence the surgical margin?

Kwon et al demonstrated that PSM rats are comparable in tumors with low malignancy (oncocytoma, angiomyolipoma, papillary RCC type I and chromophobe RCC) and tumors with high malignancy (collection duct carcinoma, sarcomatoid change, ccRCC and papillary RCC type II). So tumor histology was not predictive for PSM. However, only the tumors with high malignancy tend to recur and metastasize. The authors concluded that patients with highly malignant tumors are higher at risk to develop local recurrences of the tumor and to die of tumor related causes. (273)

How thick should the healthy parenchyma surrounding the tumor be?

Historically, a 1cm rim of healthy kidney tissue was recommended to assure complete removal of the RCC. (274) Meanwhile, data indicates that narrower margins of regular healthy kidney tissue are sufficient. (275) In a few studies simple tumor enucleation even yielded oncologic results comparable to partial nephrectomy, but no prospective trial has been performed for this propose. (267)

Do PSMs correlate with tumor reoccurrence and survival?

Yossepowitch et al. performed one of the largest studies regarding PSM, tumor survival and tumor recurrence. They found that positive surgical margins in partial nephrectomy specimens do not imply an adverse prognosis. (272) However, other investigators reported a correlation between PSM and higher rates of tumor reoccurrence rates. In summary, PSMs after PN harbor an increased risk of disease reoccurrence, especially in patients with high malignant tumors. However the vast majority of patients with PSM are not affected by local or metastatic tumor reoccurrence. (267)

Frozen section analysis: is it useful in the diagnosis of PSM?

The main problems of FS are the intermittent false-negative or inconclusive results that did not correlate with the final paraffin-section histology. (276) Sterious et al. compared patients where FS during PN was performed to patients without FS. In patients with a documented malignant tumor of the kidney PSMs were comparable ($p = 0.16$) and showed no significant difference. (277) The correct diagnosis in FS is also impeded by sending too small tissue samples to the pathologist. Also tumor-bed biopsies are not very accurate. They represent only a small fraction of the resection margin and deliver unreliable results. In conclusion, frozen section analysis seems not to deliver additional information of clinical significance and failed to impact the final margin status. (267, 277, 278)

3.2 Frozen section and diagnosis of tumor behavior (benign versus malignant)

Regarding frozen section and tumor behavior of renal masses I was not able to find any literature.

However, from other tumors we know big discordances between frozen section and paraffin histology. (283) Therefore we may suggest that there is also discordance in RCC, which is not known yet.

III. Chapter: Accordance between frozen section diagnosis and paraffin section

A retrospective clinicopathological study using a database with 3166 cases of renal lesions

1. Introduction

There are an estimated 200,000 newly diagnosed cases of renal cell carcinoma (RCC) and over 100,000 deaths caused by RCC each year in North America, Europe and Australia. (280) Historically, the gold-standard for treatment of renal cell carcinoma has been radical nephrectomy as described by Robson (264) However, during past decades due to the development of modern imaging techniques, kidney tumors are detected at earlier stages and partial nephrectomy has gained acceptance in the treatment of small renal masses. Partial nephrectomy has been shown to provide cancer free survival comparable to radical nephrectomy and the advantage of a reduced risk of cardiovascular events that directly translates into improved overall survival. (198, 199) The main concern about partial nephrectomy is the risk of local tumor reoccurrence. In accordance, long-term results show that partial nephrectomy is associated with low rates of local tumor reoccurrence. (267)

Intraoperative frozen section analysis of specimens obtained from the normal looking adjacent kidney parenchyma is commonly performed during partial nephrectomy at our institution. In case of positive surgical margins the surgeon has to extend the resection or perform a radical nephrectomy. In this study I retrospectively investigated the accordance between the yields of frozen section analysis performed during partial nephrectomy and the yields of final pathology after embedding the specimens in paraffin. Furthermore, I evaluated the rates of wrong diagnosis of histological types.

2. Material and Methods

Between 1984 and 2013, 3140 patients received renal surgery at our department of urology due to a renal cell carcinoma. After the analysis of all patients we excluded all patients which were operated between the year 1984 to 1993 (904 patients), because of incomplete data with regard to pathological diagnosis and frozen section analysis.
Between 1994-2013, 378 men and 189 women (total 567) with a mean age 60.8 years (SD = 11.7years) underwent intended partial nephrectomy at the department of urology, Medical University Graz. All patients underwent preoperative staging and in all cases, partial nephrectomy was found to be technically feasible. Only patients who did not show any sign of metastases at time of surgery and patients with with the final diagnosis of renal cell carcinoma were included. Mean tumor size was 3.3cm (SD = 1.8cm). 492 patients were operated with an elective intention and 75 patients were operated with an imperative intention.
Partial nephrectomy patients, based on their receipt of intraoperative frozen section analysis, were stratified into two groups: “frozen section analysis”-group and a “non frozen section analysis”-group. 442(78%) (295 males and 147 females) received frozen section and 125 (22%) (83 males and 42 females) did not receive frozen section analysis. Both groups were compared by using ANOVA, χ^2-test and Mann–Whitney U test.

Surgical technique

Surgical technique was the same in the “frozen section analysis”-group, performed via a retroperitoneal approach with a flank incision. With regard to open partial nephrectomy, the renal pedicle was dissected, and the renal vessels were exposed and isolated for vascular control. Gerota´s fascia was incised to expose the kidney, leaving in situ the fatty tissue surrounding the tumor. The tumors were resected with a rim of macroscopically tumor-free kidney parenchyma. Frozen section analysis was performed immediately after tumor resection. The specimens for frozen section analysis were either taken from the adjacent kidney tissue or the complete tumor was sent to frozen section, dependent on surgeon’s decision. Finally, remaining specimens were fixed with formalin and immediately transported to the pathologic institute for subsequent histological examination.

The parenchymal margin was considered “complete”, if a continuous ring of tumor-free parenchyma surrounded the lesion and “incomplete” when the tumor abutted the intact enucleation capsule.

In the “non-frozen section analysis”-group, either open partial nephrectomy or laparoscopic partial nephrectomy was performed. Our current laparoscopic technique is a transperitoneal approach with transient atraumatic clamping of the renal artery and vein, tumor excision with an as narrow as possible healthy margin using bipolar electrocautery, sharp excision with scissors, pelvicaliceal suture repair if necessary, and suture repair of the renal parenchymal defect over surgical bolsters.

In order to evaluate the accordance between frozen section analysis and the final histology, a variable called “matching” was implemented to the “frozen section analysis”-group. “Matching” was considered as 0, when frozen section and final histology did not match. Differences were noted in the surgical margin status and the histological tumor type. The “matching” and “non matching” group were also compared with the χ^2-test

The pathologic data was obtained from the Institute for Pathology at the Medical University of Graz and was submitted via the hospital internal program MEDOCS©. The pathology reports from the frozen section and permanent sections were reviewed the data was collected and statistically analyzed.
For statistical analysis IBM© SPSS© Statistics Version 20 and 21 via the following internet page using my “medunigraz”- account:
https://citrix.medunigraz.at/Citrix/XenApp/auth/loggedout.aspx?CTX_MessageType=INFORMATION&CTX_MessageKey=SessionExpired

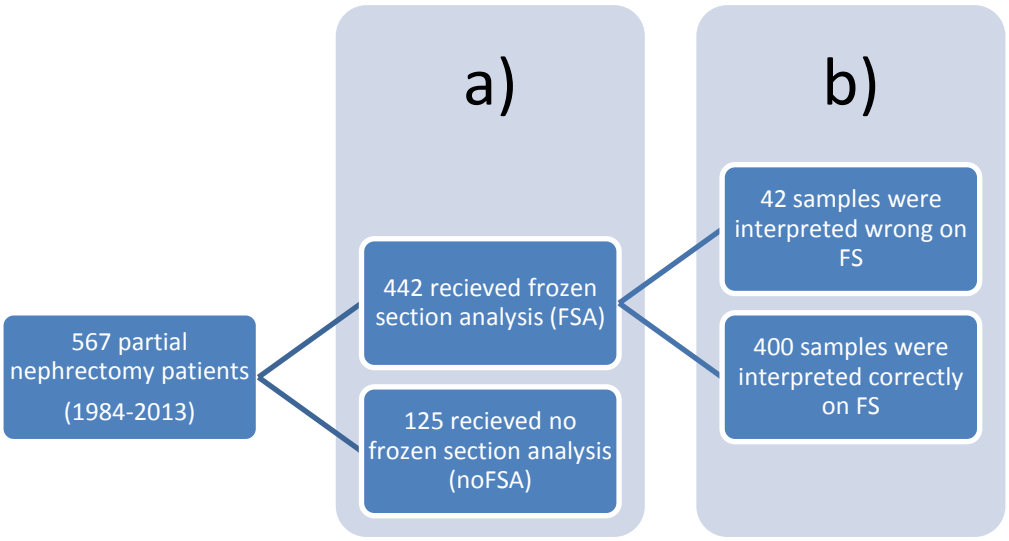

Figure III -1: Summary of my methods

A) Is frozen section analysis during partial nephrectomy necessary? First I stratified all partial nephrectomy patients in two groups "FSA"-group and "non FSA"-group and compared these two groups. Purpose of this step was to verify if there are any advantages in the use of frozen section analysis during nephron-sparing surgery.

B) How reliable is frozen section analysis? In this second step I focused my investigations on the reliability of frozen section results compared with the final paraffin histology.

3. Results

4.1 Frozen section analysis in partial nephrectomy

4.1.1 Patient characteristics

Patient demographics "FSA"-group	
Patients (n)	442
males	295 (67%)
females	147 (33%)
Age at surgery (yr)	
Mean	60.5
Median	62
SD	12
Indication for surgery	
elective	405 (91%)
imperative	37 (9%)

Patient demographics "non FSA"-group	
Patients (n)	125
males	83(66%)
females	42 (34%)
Age at surgery (yr)	
Mean	62.6
Median	63
SD	10.5
Indication for surgery	
elective	87(69%)
imperative	38 (31%)

Table III - 1: Patients demographics in "FSA"-group and "non FSA"-group

A total of 442 patients underwent partial nephrectomy with frozen section analysis. Indication for partial nephrectomy was imperative (solitary kidney, bilateral masses or renal insufficiency) in 37 (9%) patients and elective (normal renal function, normal contralateral kidney) in 405 (91%) patients.

Gender and indication for surgery are both nominally scaled relative large samples, so I have chosen the χ^2-test to compare "FSA" and "non FSA"- group. Gender did not show any significant difference in both groups (p = 0. 94). However, indication for surgery revealed a significant difference in the χ^2-test in favor for the imperative indication group. (p = 0.004) in the "nonFSA"-group.

Age is not a normally distributed variable, which could be shown due to the Kolmogorov-Smirnov test (p = 0.002 for "FSA"-group and p = 0.000 for "non FSA"-group) and the Shapiro-Wilk test (p = 0.017 for "FSA"-group and p = 0.000 for "non FSA"-group). Both showed no significance which reveals no normal distribution (parametric variable) of the parameter "age". Because of this normal distribution I have chosen the independent samples Mann-Whitney-U test. The Mann-Whitney-U test revealed no significant difference . (p = 0.087). The distribution of age is the same across categories of FSA.

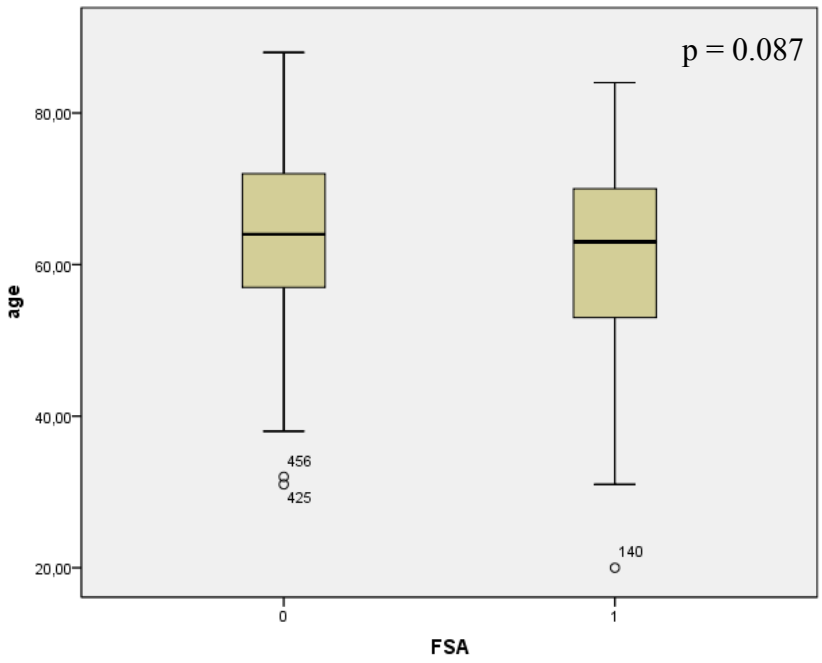

Figure III - 2: Boxplot of the age distribution in the "FSA" and "non-FSA"-group
There is no difference between both group (p = 0.087). "0" means no frozen section and "1"means frozen section during partial nephrectomy.

4.1.2 Tumor characteristics

Tumor characteristics "FSA"-group (n=442)		***Tumor characteristics "non FSA"-group (n=125)***	
Tumor size (cm)		*Tumor size (cm)*	
median	3.3	median	3.3
SD	1.8	range	1.9
Side (n)		*Side (n)*	
left	217 (49%)	left	65 (52%)
right	225 (51%)	right	60 (48%)
Pathologic diagnosis		*Pathologic diagnosis*	
ccRCC	321 (73%)	ccRCC	91 (73%)
pRCC	96 (21%)	pRCC	25 (21%)
chRCC	22 (5%)	chRCC	6 (5.25%)
cdRCC	2 (0.6%)	cdRCC	2 (0.5%)
unclass. RCC	1(0.3%)	unclass. RCC	1 (0.25%)
pT-classification		*pT-classification*	
T1a	332 (75%)	T1a	91 (73%)
T1b	57 (13%)	T1b	18 (14%)
T2a	13 (3%)	T2a	4 (3%)
T2b	1 (0.25%)	T2b	1 (1%)
T3a	32 (7%)	T3a	9 (7%)
T3b	3 (0.75%)	T3b	1 (1%)
T3c	1 (0.25%)	T4	1 (1%)
T4	3 (0.75%)		
Grade (Fuhrman)		*Grade (Fuhrman)*	
G1	150 (33%)	G1	45 (36%)
G2	242 (54%)	G2	69 (51%)
G3	54 (12%)	G3	11 (13%)
G4	2 (1%)		
Vascular invasion		*Vascular invasion*	
no invasion	410 (93%)	no invasion	120(96%)
microscopic	21 (5%)	microscopic	3 (2.4%)
macroscopic	11 (2%)	macroscopic	2 (1.6%)
Sarcomatoid diff.		*Sarcomatoid diff.*	
no	439 (99.2%)	no	123 (98.4%)
yes	3 (0.8%)	yes	2 (1.6%)

Table III -2: Tumor characteristics in “FSA”-group and “non FSA”-group

Shapiro-Wilk test (p = 0.000 for “FSA”-group and p = 0.000 for “non FSA”-group) and Kolmogorov-Smirnov test (p = 0.000 for “FSA”-group and p = 0.000 for “non FSA”-group) revealed a significant difference between the distribution of tumor size and normal distribution.

Hence, tumor size is a non parametric variable. Further tumor size was compared between ”FSA”-group and “non-FSA”-group with the independent samples Mann-Whitney-U test. Mann-Whitney-U test showed no significant differences between “FSA” and “nonFSA”-group. (p = 0.515)

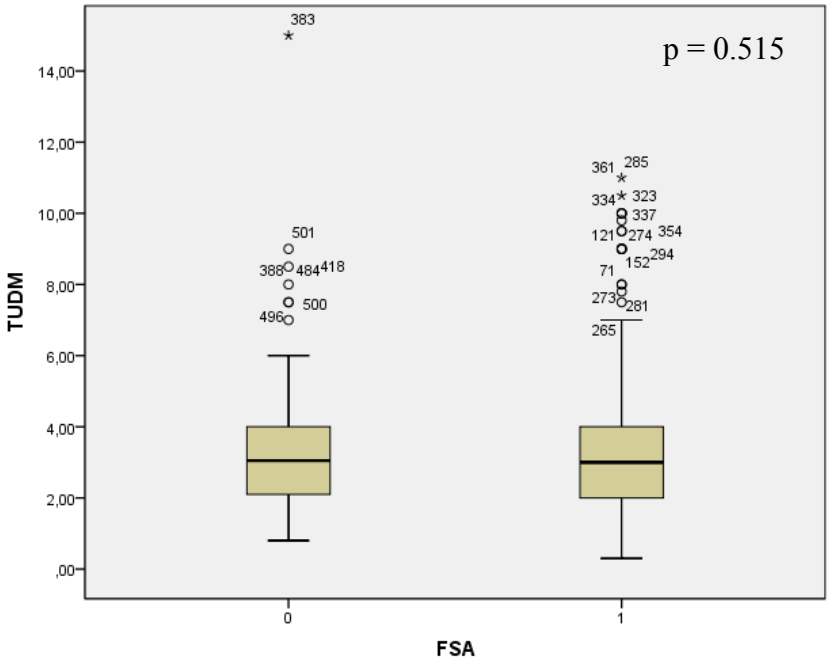

Figure III - 3: Boxplot of the tumor size in the “FSA” and “non-FSA”-group

There is no difference between both group (p = 0.065). “0” stands for no frozen section and “1”stands for frozen section during partial nephrectomy.

Tumor characteristics including side (p = 0.5), size (0.065), pT-classification (0.87), histological subtypes (0.46), Fuhrman grade (0.66), presence of vascular invasion (0.28), and sarcomatoid changes (0.60) did not show statistically significant differences between FS and non-FS subgroups

Positive surgical margins

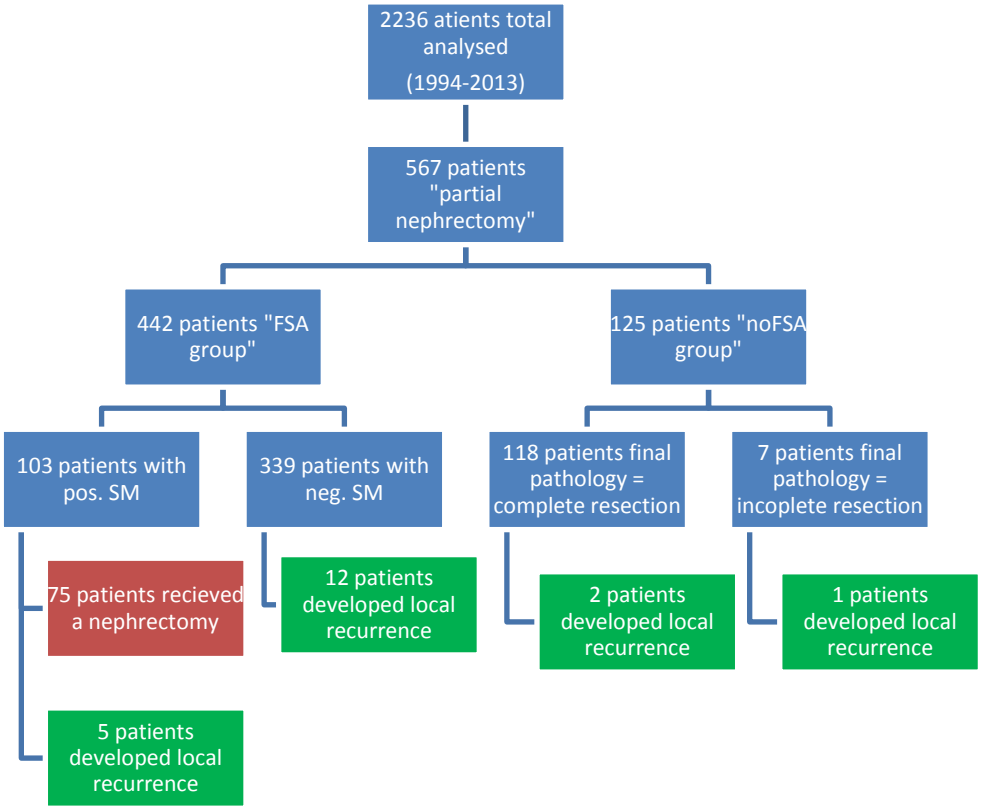

Figure III - 4: Intraoperative examination of frozen section and local tumor recurrence
This figure shows the distribution of the local tumor recurrence. Green = local tumor recurrence ; Red = these patients received an immediate nephrectomy because of the PSM in the frozen section.

Frozen sections analysis yielded positive surgical margins (PSM) in 103 patients (23.3%) of the 442 patients in the "FSA"-group. Out of these 103 patients with a PSM in the frozen section, 35(34%) patients were confirmed as PSM by paraffin embedded histology (true positive in frozen section). In contrast, 68(66%) patients had negative surgical margins in paraffin embedded histology (false positive in frozen section). Out of the 339 patients with a negative surgical margin in frozen section 327 (96.5%) patients were confirmed as negative surgical margins by paraffin embedded histology(true negative in frozen section). whereas in 12 cases margins were positive in the final histology reports false negative in frozen section). In the "nonFSA"-group final paraffin histology yielded PSM in 7 (5.6 %) patients of 125 patients who did not receive intraoperative frozen section analysis during partial nephrectomy.

Frozen section analysis was discordant to the paraffin embedded histology as gold standard of histological diagnosis in 80/442 cases. (18%) These are 68 false positive and 12 false negative results.

In case of a PSM in frozen section the surgeon extends the resection if technically feasible or performs a nephrectomy and removes the whole kidney. Negative resection margins lead to the completion of the operation.

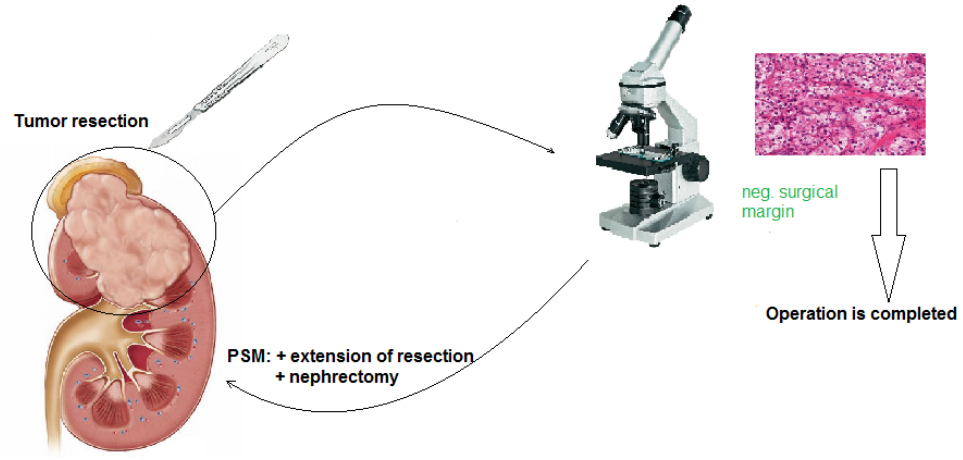

Figure III - 5: Procedure of frozen section

75 (73%) of the 103 patients with PSM received a total nephrectomy because of a positive margin in frozen section. In 22/75(30%) of these patients a residual tumor was confirmed by paraffin embedded histology, whereas 53/75 kidneys (70%) were tumor-free after nephrectomy in final histology. These nephrectomy patients were removed from any further statistical analysis regarding local recurrence, since this endpoint could not be met after radical nephrectomy. Thus, FSA altered the surgical intervention in 75/442 (17%) operations. In 22/442 (5%), the switch to radical nephrectomy was justified because of residual tumor in the specimen confirmed by paraffin embedded histology. In contrast, FSA resulted in overtreatment in 53/442 (12%) patients, who lost tumor-free kidneys. Final margins were positive in 7 /125 patients (5.6%) in the “nonFSA” group compared to 47 /442 patients (10.6%) in the “FSA”group (χ^2-test p = 0.091). This statistical analysis did not reveal a significant difference between incomplete tumor resection with regard to performance of intraoperative frozen section analysis

The remaining 28 patients with a PSM in frozen section received an extension of the tumor resection until the margins were confirmed as negative. The patients with a residual tumor in the extended resection were counted as true positive the others as false positive.

After exclusion of 75 radical nephrectomy patients, 20/492 (4%) remaining patients developed a local recurrence after partial nephrectomy.

After exclusion of the radical nephrectomy cases (442– 75nephrectomy patients), 17/367 (4.6%) patients developed local tumor recurrencein the FSA-group.5 of these patients had a PSM in the frozen section. The PSM was confirmed in 3 patients by paraffin embedded histology. The other 12 patients had a negative surgical margin in the frozen section. 11/12 patients had a confirmed negative surgical margin in paraffin embedded histology. 1/12 had a PSM in the final pathology.

In the non-FSA-group 3/125 (2.4%) patients developed a local recurrence. Surgical margins were negative in 2 and positive in one of these patients in the final pathology report.
Overall, out of 20 patients developing local recurrence, 5 (25%) had confirmed positive margins in the final histology after partial nephrectomy, whereas margins were negative in 15 (75%). In our whole study population, final margins were positive in 54/567 (9.5%) patients.

75 operations were started with the intention of nephron sparing surgery and intraoperatively switched to a total nephrectomy because of the frozen section results.
75 kidneys were completely removed because of a PSM in frozen section and analyzed via paraffin section. The results of these analysis yielded that only 22 (30%) of the 75 removed kidneys showed a residual tumor in the kidney. So, the diagnosis of positive margins in frozen section was true positive in 22 and false positive in 53patients, respectively.

These results permit to calculate the sensitivity, the specificity the positive and negative predictive value.

		Paraffin embedded histology		
		positive	negative	overall
frozen section	positive	35	68	103
frozen section	negative	12	327	339
	overall	47	395	442

Table III - 3: Crosstable Sensitivity, Specificity, pos. and neg. predictive value

Sensitivity, Specificity, pos. and neg. predictive value of frozen section compared with paraffin embedded section regarding positive surgical margins

Sensitivity:	74.5%
Specificity	82.8%
false-Positive:	17.2%
false-Negative:	25.5%
pos. predictive value:	34.0%
neg. predictive value:	96.5%
Overall accuracy:	0,82

Table III - 4: Results from the crosstable

The calculation yields a low sensitivity and a high specificity for frozen section analysis in renal lesions. The low positive predictive value of this diagnostic procedure suggests that frozen section analysis is not a reliable diagnostic test to predict residual tumor masses after partial nephrectomy.

The central reason for using intraoperative frozen section is to avoid local recurrence of the tumor on the base of microscopically remaining tumor masses in the kidney. Therefore χ^2-test was performed to show a difference between “FSA” and “nonFSA” in local tumor recurrence. This test did not show a significant difference in local recurrence ($p= 0.33$) Frozen section does not help to avoid local recurrence of the RCC.

In case of PSM there is a significantly higher rate (17% PSM vs. 3.5% NSM) of local recurrence compared to the negative margins, despite the extension of the resection after the positive result. (χ^2-test $p = 0.001$)

"FSA"-group metastases and survival				
surgical margin	*total patients*	*metastatic disease*	*tumor spesific death*	*follow up [days]*
pos. SM	103	11 (11%)	7 (7%)	1300
neg. SM	339	16 (5%)	2 (0.6%)	950
local recurrence	17	6 (35%)	3 (17%)	1300

Table III -5: "FSA"-group metastases and survival

In the “FSA”-group 11 of the 103 patients with a PSM during partial nephrectomy developed metastatic disease and 7 died by virtue of the tumor within a mean follow up of 43.7 months (± 30.2 months). In the group of patients with a negative surgical margin 16 of 342 patients developed metastatic disease and 2 died on the basis of tumor specific complication within a mean follow up 31.2 months (± 36.1 months).
The results of the frozen section compared with final section results are same in all patients with metastatic disease.

In the whole “FSA”-group 27 (6%) of 442 patients developed metastases and 9 (2%) of these died in the follow up. (Mean = 28.2 ± SD =36.1days)

"nonFSA"-group metastases and survival				
surgical margin	*total patients*	*metastatic disease*	*tumor spesific death*	*follow up [days]*
negative	118	6 (5%)	4(3%)	1800
positive	7	1 (14%)	0(0%)	1300
local recurrence	3	1(33%)	0 (0%)	950

Table III -6: "nonFSA"-group patients with positive surgical margins

In the “nonFSA”-group 7 of the 125 patients (6%) developed metastatic disease and 4 (3%) died by virtue of the tumor within a mean follow up of 59.1 months (± 65 months). In the group of patients with incomplete resection of the tumor in the final pathology only 1 of 7 patients developed metastatic disease.

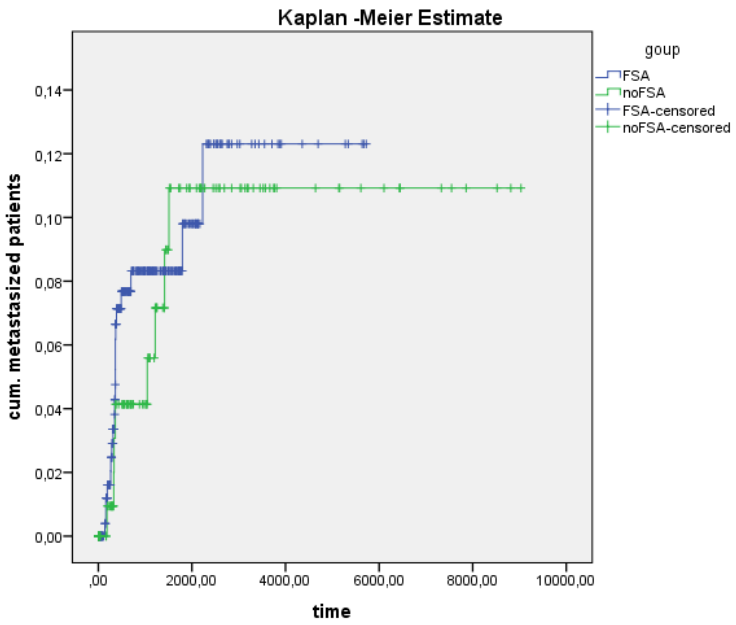

Figure III - 6: Kaplan-Meier estimate of metastasized patients

The estimate shows how many patients per group develop metastasized disease over time (days).

Log rank test (p = 0.643) revealed no significant difference between both curves. This indicates frozen section analysis in renal lesions has no influence on metastatic behavior of the RCC and the tumor specific overall survival (see the Kaplan-Meier estimate of overall survival).

To investigate the influence of frozen section analysis on survival of patients which were treated with partial nephrectomy, I performed Kaplan-Meier analysis.

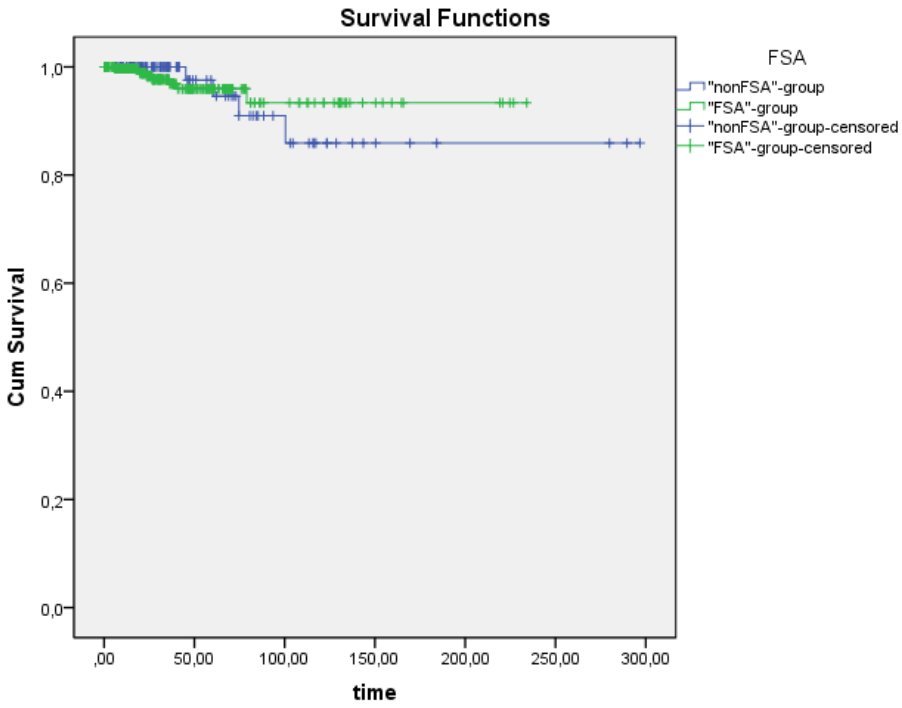

Figure III - 7: Kaplan-Meier estimate of the cancer specific survival

Kaplan-Meier analysis revealed that frozen section analysis did not considerably contribute to preventing from tumor specific death. (log rank p = 0.863)

4.2 How reliable is frozen section analysis regarding histological tumor entities?

The average renal tumor size was 3,5cm (range 0,8 to 15cm). The median age for all patients was 63 years (range 20 – 84 years).

Diagnoses by frozen section of renal tumors were discordant with final pathology in 42 of the 400 patients (10.5%). In discordant cases, overdiagnosis occurred in 4 (1%) cases, which were diagnosed as ccRCC, adenocarcinoma of the gastrointestinal tract and Lymphoma on frozen section. Under-diagnosis occurred in 38 cases (99%) at time of frozen section; 6 pRCC, 2 chRCC, 4 ccRCC and one ccRCC were interpreted as oncocytoma; 3 pRCC and 10 multilocular cystic RCC were interpreted as benign cysts; 3 pRCC and 2 ccRCC were interpreted as renal adenoma; 2 ccRCC were interpreted as angiomyolipoma; 1 chRCC was interpreted as amyloidal infiltration; 1 ccRCC was interpreted as hemangioma; 1 liposarcoma was interpreted as pRCC; 1 chRCC and 1 oncocytoma were interpreted as ccRCC and 2 pRCC were interpreted each as inflammation and ganglion on frozen section.

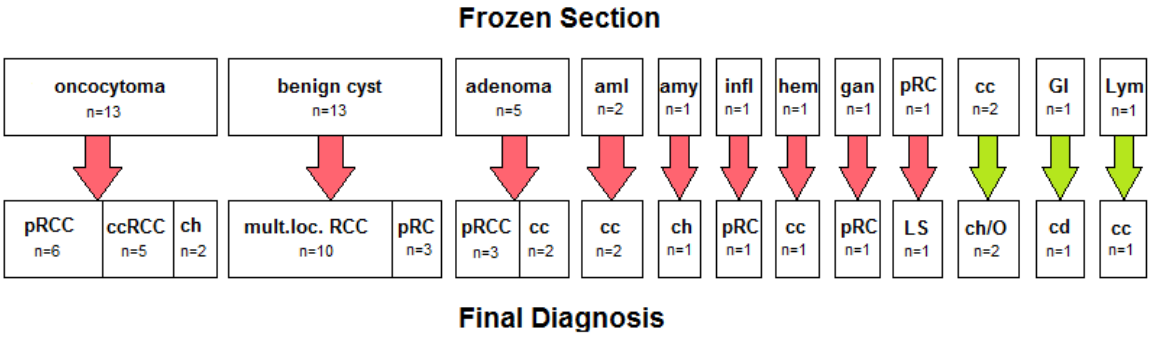

Figure III - 8: Discordant cases between frozen section and final pathology diagnosis
Aml = angiomyolipoma, amy = amyloidal infiltration, infl = inflammation, hem = hemangioma, gan = ganglion, pRC = papillary RCC, cc = clear cell RCC; GI = gastrointestinal adenocarcinoma, lym = lymphoma, ch = chromophobe RCC , mult.loc. RCC = multilocular cystic RCC, LS =Liposarcoma, ch/O = chromophobe RCC and Oncocytoma, cd = collecting duct carcinoma of Bellini, green arrows stand for over diagnosed, red arrows stand for under diagnosed (n=42)

With regard to the diagnosis of oncocytomas and benign renal cysts, there was a high discordance between the frozen section and the final pathology. All 13 tumors, diagnosed as oncocytomas in the frozen section, were upgraded to malignant tumors in the final pathology report. (6 pRCC, 2 chRCC, 5 ccRCC) The same was true for all 13 tumors, primarily diagnosed as benign cysts. (10 multilocular cystic RCC, 3 pRCC).

Despite of 11% (42 patients) cases being discordant on frozen section and final pathology, a clinical impact was noted only in 1 case, where a liposarcoma was misinterpreted as pRCC on frozen section.

Univariable analysis did not reveal any association with the accuracy of frozen section diagnosis and patient age, tumor size, presence or absence of sarcomatoid differentiation, the side of tumor localization, grading or staging. (Table III-5)

Characteristic	***Frozen section compared with final diagnosis***			
	underdiagnosed (n=4)	overdiagnosed (n=38)	same diagnosis (n=400)	overall p
Age				
≤ 50	0	6	91	0,332
> 50	4	32	309	
Size				
≤ 4cm	4	31	312	0,997
> 4cm	0	7	88	
Sarkomatoid differentiation				
absence	3	37	398	0,402
presence	0	1	2	
Side				
left	2	14	194	0,34
right	2	24	206	
Grade (Fuhrman)				
G1	1	21	132	0,134
G2	1	12	219	
G3	1	5	46	
G4	0	0	3	
Stage				
T1a	2	30	304	0,514
T1b	0	2	61	
T2a	0	2	2	
T2b	0	0	3	
T3a	1	3	24	
T3b	0	0	2	
T3c	0	0	1	
T4	0	1	3	

Table III - 7: Univariate analysis for predictors of discordance

4. Discussion

Nephron- sparing surgery provides a lower risk of chronic kidney disease which can be translated into a reduced risk for cardiovascular events and an improved overall survival in comparison with radical nephrectomy. (284, 285, 286) However, the risk of incomplete tumor removal is of concern with nephron-sparing surgery. Therefore, frozen section analysis during partial nephrectomy is routinely performed at many institutions. Findings of positive surgical margins usually imply commitment to extension of the surgery by wider resection or conversion to radical nephrectomy. However, the risk of local disease recurrence or metastatic progression couldn't be observed in one of the largest studies on patients with positive surgical margins after partial nephrectomy. (272) According to this study a wider resection or radical nephrectomy may result in an unnecessary loss of normal renal parenchyma, as well as lengthened surgical time and increased costs. Based on the whole FSA-group of 442 patients in our study, the positive predictive value of surgical margins obtained by frozen section was only 34% and resulted in unnecessary radical nephrectomies in 53/442 (12%) patients, On the other hand, usage of FSA provided clinical benefit by prevention of local recurrence in those 22 (5%) patients who had residual tumor after radical nephrectomy.Venigalla et al. retrospectively analyzed 433 patients undergoing partial nephrectomy. They found a significantly decreased rate of positive surgical by performing frozen section analysis. However, they found no significant difference in the rate of local tumor recurrence in patients undergoing open surgery. (278)

Sterious et al. investigated 537 patients undergoing partial nephrectomy and found no significant difference in the rate of positive surgical margins in patients who received frozen section analysis and those who did not. (277)

Duvdevani et al. reported that although frozen section is routinely performed only 2 patients of 301 had PSM and no tumor was found after nephrectomy of these patients. This study group could also demonstrate that the tumor was observed in paraffin section in 4 cases with a negative surgical margin. (276) These results emphasize our finding of the low sensitivity and the high specificity of the diagnostic procedure frozen section analysis in renal lesions. In our cohort 53 out of 75 nephrectomies were performed without a sign of a remaining tumor in the kidney. We could also show that the routinely performance of frozen section does not influence the treatment result like local tumor recurrence, metastases or the overall survival. Based on these result, especially the low sensitivity, it seems that the macroscopic assessment of the tumor bed by an experienced surgeon may

be sufficient. However, a small number of cases are the limitation of these finding. Although frozen section promotes a greater confidence for the surgeon, but the routinely application should be investigated more extensively.

5. Conclusion

In this big single-center cohort, frozen section analysis failed to have an impact on both local recurrence and clinical outcomes. Oncological outcomes regarding progression to metastatic disease and tumor related death were comparable between both groups. Use of frozen section resulted in prevention of local recurrence by nephrectomy for residual tumorin 5%, but in overtreatment by radical nephrectomy of tumor-free kidneys in 12%. Our study revealed a low positive predictive value for frozen section regarding margin status in partial nephrectomy. Consequently, we conclude that routinely frozen section analysis during partial nephrectomy does not provide any benefit with respect to oncological outcomes and may even result in potentially harmful overtreatment in a clinically relevant subset of patients.

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