Interleukin-2 based immunotherapy in patients with metastatic renal cell carcinoma

Frede Donskov

This review has been accepted as a thesis together with eight previously published papers, by the University of Aarhus, November 28, and defended on August 31, 2007.

Department of Oncology Aarhus University Hospital and Institute of Medical Microbiology and Immunology, Aarhus University, Århus, Denmark.

Correspondence: Islandsgade 9, 8200 Århus N, Denmark.

E-mail: fd@microbiology.au.dk

Official opponents: Pieter H. De Mulder, Mogens Claesson and Jens Overgaard.

Dan Med Bull 2007;54:249-65

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignancy (85%) of the kidney. The remainders are mainly transitional cell carcinomas originating from the renal pelvis. Willms' tumor (nephroblastoma) is only seen in children. In the following, emphasis is restricted to RCC.

EPIDEMIOLOGY

RCC accounts for 2 percent of all cancer. Worldwide, 208,000 new cases and 102,000 deaths result each year from renal cancer [1]. The incidence varies among countries. The rates are highest in Western and Eastern Europe, North America, Australia and Scandinavia, intermediate in Southern Europe and Japan, and low elsewhere in Asia, South America, Africa and the Pacific [2]. In the United States, the incidence has increased by 43% since 1973, and similarly, increased incidence rates have been observed in nearly all regions. By contrast, in Denmark and Sweden the incidence rates have remained almost unchanged [3]. The absolute numbers of new kidney cases and kidney cancer deaths in Denmark during the last 30 years are seen from Figure 1.

Risk factors for developing RCC include cigarette smoking – the proportion of RCCs that could be attributed to cigarette smoking is op to 30% – hypertension, obesity and end-stage renal disease [3-5]. The median age at diagnosis is 65. The men-female ratio is 2.5:1.

PATHOLOGY, GENETICS AND TUMOR BIOLOGY

International agreement was reached in 1997 on the histologic classification of RCC on the first international multidisciplinary workshop held by World Health Organization (WHO) in collaboration with the Union Internationale Contre le Cancer (UICC) and American Joint Committee on Cancer (AJCC) [6, 7]. Thus, RCC are now separated into four different cellular types: clear, papillary, chromophobe and collecting duct (Table 1). The most common histological type of RCC is clear cell carcinoma (75% of cases). These tumors arise from the proximal tubule in the renal cortex (Table 1). This tumor type is typically sporadic, unilateral and unifocal. A large proportion of these tumors have mutations or hypermethylations at chromosome 3p, which contains the von Hippel-Lindau tumor suppressor gene. Papillary tumors occur in 15% of cases, and are the next most common histological type. Cytogenetically, these tumors frequently have trisomy of several chromosomes, as well as loss of the Y chromosome (Table 1). There are two subtypes of papillary RCC, type 1 (small cells) and type 2 (large cells with abundant eosinophilic cytoplasm) [8].

Inherited RCC is rare, only up to 2% of RCC cases cluster in families [9]. Within the last decade, four genes leading to inherited forms of RCC have been identified [10]; the von Hippel-Lindau (VHL) gene leading to clear cell RCC [11] was identified on chromosome 3 in 1993; the c-Met gene leading to type 1 papillary RCC [12] was identified on chromosome 7 in 1997; the Fumarate hydratase (FH) gene leading to Type 2 papillary RCC [13] was identified in 2002; and the Birt-Hogg-Dubé (BHD) gene leading to chromophobe RCC [14] was identified in 2002.

During the past decade, the biology associated with the VHL gene product (pVHL) has been elucidated in inherited and sporadic RCC [8, 15]. The von Hippel-Lindau tumor suppressor gene (VHL), which resides on chromosome 3p25, is mutated or silenced in 100% of inherited and 50-85% of sporadic clear cell RCC. Mutation or methylation of VHL leads to a pseudohypoxic state in which the pVHL complex does not form and/or cannot degrade hypoxia-inducible factors (HIF-1α, HIF-2α and HIF-3α). Thereby, HIF overaccumulates resulting in increased transcription of a variety of genes, including vascular endothelial growth factor (VEGF), platelet-derived growth factor B (PDGF-B), transforming growth factor-α (TGF-α), Glut 1 glucose transporter, carbonic anhydrase IX and erythropoietin (EPO) [8, 16]. Thus, VHL appears to be a critical gatekeeper for the development of clear cell RCC and for the subsequent tumor proliferation, angiogenesis, mitogenesis, erythropoiesis, glucose metabolism, and pH control [15, 16].

The grading of RCC began in 1932, reflecting the differentiation of the tumor cells as defined microscopically by increased nuclear size, irregularity, and nucleolar prominence [17]. Since then, several histopathological grading systems have been proposed, but currently that proposed by Furhman et al [18] is the most widely used in the North America and that proposed by WHO is most widely used in Europe [17].

CLINICAL PRESENTATION

In the onset of RCC, there are only few early warning signs. The classical triad of hematuria, flank pain and abdominal mass is found in less than 10% [4]. Among patients who are symptomatic, more than 50% have hematuria, approximately 40% have pain, 40% have an abdominal mass, and 10% have symptoms arising from metastatic sites. Patients also frequently have nonspecific signs and

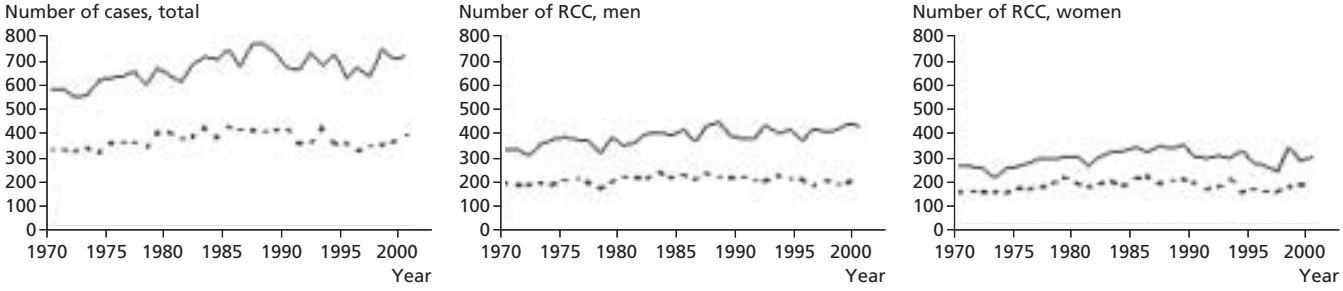


Figure 1. Incidence (—) and mortality (--) of RCC in the period 1970-2000; overall (left); in men (middle); and women (right).

Table 1. Renal cell carcinoma. Putative cells of origin, frequency and genetic correlates.

Tumor type	Putative cell of origin	Frequency	Genetic abnormalities
Clear cell RCC (Conventional)	Proximal convoluted tubule	75%	3p- VHL genemutation
Papillary RCC	Distal convoluted tubule	15%	3+, 7+, 12+, 16+, 17+, Y- *c-Met* genemutation FH genmutation
Chromophobe RCC	Intercalated cells, cortex	5%	1-, 2-, 6-, 10-, 13-, 17-, 21-, Y- BHD genemutation
Collecting duct RCC	Collecting duct	1%	1-, 6-, 14-, 15-, 22-
Unclassified RCC		4%	

Data from [6, 7]. Abbreviations: VHL, Von Hippel Lindau; FH, Fumarate hydratase; BHD, Burt-Hogg-Dubé.

symptoms such as weight loss, fever, malaise, hypercalcemia, anemia or erythrocytosis.

Metastatic disease is present in 30% of patients at initial diagnosis. Among these patients, approximately 75% have lung metastases, 36% have lymph node and/or soft tissue metastases, 20% have bone metastases, 18% have liver metastases, and 8% each have skin and CNS metastases [4, 19]. Moreover, metastatic disease will develop in 30-50% of patients relapsing after initial curative-intended nephrectomy. As a result, approximately 50% of RCC patients will suffer from metastatic disease [19].

STAGING

Historically, several staging systems have been used in parallel. The system proposed by Robson et al in 1963, and updated in 1969, was most commonly used in clinical practice [20]. However, the first international multidisciplinary workshop held in 1997 by World Health Organization in collaboration with the Union Internationale Contre le Cancer (UICC) and American Joint Committee on Cancer (AJCC) [21], led to the general acceptance of the TNM classification system as one single internationally staging system for RCC (Table 2). In 2002, an update was published, subdividing stage T1 into T1a and T1b on the basis of a 4.0 cm cut-off level.

Table 2. TNM staging of RCC [21].

Primary tumor (T)			
T1a	Tumor ≤ 4 cm, limited to the kidney		
T1b	Tumor > 4 cm but ≤ 7 cm, limited to the kidney		
T2	Tumor > 7 cm, limited to the kidney		
T3a	Tumor invasion of adrenal gland or perinephric tissues		
T3b	Tumor extension into renal veins or the vena cava below the diaphragm		
T3c	Tumor extension into the vena cava above the diaphragm		
T4	Tumor extension beyond Gerota's fascia		
Regional lymph nodes (N)			
N0	No lymph node metastases		
N1	Metastasis to one regional lymph node		
N2	Metastasis to more than one regional lymph node		
Distant metastasis (M)			
M0	No distant metastasis		
M1	Distant metastasis		
Stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1 or T2	N1	M0
	T3	N0, N1	M0
Stage IV	T4	N0, N1	M0
	any T	N2	M0
	any T	any N	M1

PROGNOSIS

The 5-year survival probability for RCC is approximately 95% for stage I patients, 88% for stage II patients, 59% for stage III patients, and 20% for stage IV patients [22].

For the subset of stage IV patients with untreated metastatic (M1) disease, the prognosis is poor as the median survival is only 8 months [23], the overall 3-year survival is less than 10% [24, 25], and the 5-year survival is less than 2% [26]. This dismal prognosis has not changed significantly in the past 30 years [27].

TREATMENT OF LOCALIZED DISEASE

The principal treatment for non-metastatic RCC is radical nephrectomy, which includes excision of the kidney with all of the Gerota's fascia, removal of the ipsilateral adrenal gland and regional lymphadenectomy [28]. An eventual intracaval tumor thrombus should be resected [29]. The actual benefit derived from adrenalectomy and lymphadenectomy has been debated and most centers now reserve adrenalectomy for patients with large upper-pole lesions and lymphadenectomy for patients with abnormal-appearing abdominal lymph nodes on computed tomography or clinically during surgery [30, 31-34]. Laparoscopic and partial nephrectomy [35] and radiofrequency ablation [36] are presently being evaluated and incorporated into clinical practice.

There is no indication for adjuvant therapy, after resection for localized RCC, outside clinical trials. Studies of radiation therapy to the renal bed combined with neprectomy – adjuvant as well as neoadjuvant – have shown no survival benefit, but, in contrast, a trend for a harmful effect with the expense of significant toxicity (Table 3).

Adjuvant systemic therapies have been either ineffective in terms of overall survival or even harmful compared with observation alone (Table 4). Only the study by Jocham et al [41] showed a significant progression-free survival benefit in favor of the vaccine group. However, despite a follow-up time of minimum 4.5 years, no overall survival rates were reported. This lack of essential information compromises the study.

Table 3. Randomized trials evaluating the role of radiotherapy combined with radical nephrectomy.

Author	Timing	Dose	Number pts	5-y survival
Van der Werf-Messing [37]	Preoperative	30 Gy	64	28% (NS)
		Observation	62	28%
Juusela [38]	Preoperative	33 Gy	38	47% (NS)
		Observation	50	63%
Finney [39]	Adjuvant	55 Gy	52	36%
		Observation	48	47% (NS)
Kjær [40]	Adjuvant	50 Gy	32	50%
		Observation	33	62% (NS)

NS, non significant.

TREATMENT OF METASTATIC RCC – SURGERY

In 1939, Barnay and Churchill [47] reported the first case in which a patient underwent both nephrectomy and excision of a single pulmonary metastasis. This patient survived 23 years without recurrence. There is now general agreement that a primary kidney tumor and a simultaneous solitary metastasis should be resected with curative intent. Kovolius et al [48] from Department of Surgery, Memorial Sloan-Kettering Cancer Center, addressed the issue of whether an aggressive surgical approach should be taken in patients with recurrent disease. They reported a 54% 5-year survival rate for patients who had a curative intended resection of a solitary metastasis and a 29% 5-year survival rate for patients who had curative intended resections of multiple metastases [48]. The 5-year survival rates of patients who underwent complete resection of second and third recurrences were 46% and 44%, respectively. Thus, in case of metastatic disease, surgery should always be considered. However, a case of a solitary metastasis in RCC is rare. Most often, bulky multiple metastatic disease is seen, thereby making curative intended resection impossible.

The role of radical nephrectomy in patients with non-resectable metastatic disease is controversial. Spontaneous regression of metastatic lesions after nephrectomy have been observed in about 1% of cases [49, 50]. However, the practice of nephrectomy in mRCC, in the hope of a spontaneous regression, is not recommended [27]. The hypothesis that debulking nephrectomy prior to immunotherapy may reduce a large immunosuppressive tumor burden has prompt several retrospective non-randomized trials evaluating this issue. Beldegrun et al [51] from UCLA, Los Angeles, reported that among patients who received IL-2 based immunotherapy with their primary tumor in place (n=36), the 2-year survival rate was 4%, compared with a 2-year survival rate of 44% for patients who underwent nephrectomy prior to IL-2 (n=235) [51]. However, surgery may preclude the administration of systemic therapy in a large proportion of the patients (22%-77%) because of morbidity and/or rapid tumor progression [52-54]. The survival benefit may thus be caused by selection of patients with favourable prognostic features rather than a beneficial effect of nephrectomy per se.

Two recent randomized studies [55, 56] and a combined analysis [57] have evaluated the role of debulking nephrectomy plus interferon in mRCC (Table 5). The data from these trials should be interpreted with caution. Thus, the study by Flanigan [56] took 7 years, in 80 centers, to accrue 246 patients at a rate of one patient every 2 years per institution. This raises the concern of a possible selection bias. Only patients with excellent performance status 0 or 1 (i.e. patients with no or only few symptoms) were eligible. The mortality related to the surgical procedure was lower (1.4%) [57] than earlier reported (average 2.5%) [58]. Unfortunately, the combined analysis showed a survival benefit of only 5.8 months, a 1-year survival rate of approximately 50% and a 6-year survival rate of only 3%. Moreover, one of the main hypotheses – that patients may have a better response to IFN after removal of the large immunosuppressive primary tumor – was not supported (Table 5). Therefore, it has been suggested that the survival difference occurred because of decreased morbidity and mortality from local problems caused by the primary tumor [59]. Debulking nephrectomy followed by IL-2 has not been evaluated in randomized trials.

In Denmark, the recommendation is to consider nephrectomy (and/or removal of any residual viable tumor) only in patients who after IL-2 based immunotherapy have had a major response [34]. Furthermore, nephrectomy may be justified in patients when the intent is to improve quality of life, such as the alleviation of symptoms i.e. pain, hematuria, erythrocytosis, uncontrolled hypertension or persistent hypercalcaemia that does not respond to pharmacologic agents [27, 60].

TREATMENT OF METASTATIC RCC – RADIATION

RCC is generally radioresistant, and the indication for radiation therapy is limited. The major indication for radiation therapy is for palliation of symptomatic metastatic disease, most commonly painful bone lesions and brain metastases [28].

TREATMENT OF METASTATIC RCC – CHEMOTHERAPY

RCC is resistant to chemotherapy. Chemotherapeutic agents, either alone or in combination, have little or no effect against mRCC [28]. Yagoda et al [61] reviewed the results of 4,093 patients treated in 83 trials with 74 drugs and combinations of drugs (chemotherapeutic agents and drugs with miscellaneous activity), published between 1983 and 1993. An objective response rate of 6%, including a CR-rate of 1%, was reported. Similarly, Motzer and Russo reviewed 33 chemotherapy agents studied in 51 phase II trials, published between 1990 and 1998, comprising a total of 1,347 patients [60]. Response rates were low and moreover, responses were generally short lasting. The mechanisms of drug resistance may probably be related to the fact that 80% of cancer cells have expression of the multiple

Table 4. Randomised trials evaluating the role of adjuvant systemic therapy after radical nephrectomy.

Author	Treatment	Stage	Number pts	5-year disease free survival	5-year overall survival
Pizzocaro [42]	MPA	M0	58	67% (NS)	
	Observation		62	73%	
Pazzocaro [43]	IFN-α	II-III	123	57% (NS)	66% (NS)
	Observation		124	67%	67%
Messing [44]	IFN-αNL	III-IV	140	37% (NS)	51% (p=0.09)
	Observation		143	41%	62%
Clark [45]	IL-2	III-IV	33	32% (NS)*	80% (NS)*
	Observation	+ M1 (NED)	36	45%	86%
Atzpodien [46]	IL-2/IFN/5-FU	III-IV	135	42% (NS)	58% (p=0.028)
	Observation	+ M1 (NED)	68	49%	76%
Jocham [41]	Vaccination	I-III	177	77% (p=0.02)	not reported
	Observation		202	68%	

Abbreviations: MPA, medroxyprogesterone acetate; NED, no evidence of disease after nephrectomy + resection of a solitary metastasis; NS, non significant; *) 3-years data.

Table 5. Randomized trials evaluating the role of debulking nephrectomi.

Author	Treatment	Number pts	Response rate	Med survival (months)	1-year survival	Survival advantage
Mickisch [55]	Nx + IFN	42	19%	17 (p=0.03)	-	10 months
	IFN	43	12%	7		
Flanigan [56]	Nx + IFN	120	3.3%	11.1 (p=0.05)	49.7% (p=0.012)	3 months
	IFN	121	3.6%	8.1	36.8%	
Combined analysis [57]	Nx + IFN	161	6.9%	13.6 (p=0.002)	51.9% (p=0.001)	5.8 months
	IFN	163	5.7%	7.8	37.1%	

Abbreviations: Nx, nephrectomy; IFN, Interferon-alpha.

drug resistance gene that encodes P-glycoprotein [62]. Thus, no chemotherapy agent has produced response rates in proportions that justify its use in mRCC [60] and no agent should be considered standard of care in mRCC [63].

TREATMENT OF METASTATIC RCC – HORMONAL THERAPY

No hormone therapy has produced a response rate that justifies its use in mRCC, despite renal tumor cells express receptors for estrogen and progesterone. However, for many years there has been a tradition of using medroxyprogesterone acetate (MPA) and tamoxifene for the palliation of mRCC.

TREATMENT OF METASTATIC RCC – CYTOKINE THERAPY

The treatment of mRCC by immunologic manipulation has long been regarded as a promising approach. The basis for this view has originated from observations that metastases may regress spontaneously following nephrectomy [49, 50, 64-67], late relapses after nephrectomy and prolonged stabilization of metastatic disease in the absence of systemic treatment [65]. Evidence for an immune response against RCC is provided by the observations that tumor-infiltrating lymphocytes can be detected in RCC tissues [68]. Overall, spontaneous regression occurs in 1% of patients [4]. These observations have led to considerable interest in research into immunologic therapies, as this phenomenon is considered immune mediated.

Some early attempts to induce a non-specific immune response to cancer involved "Coley's toxin", a crude extract of streptococcus [69] as well as regimens of crude tumor cell preparations, bacillus Calmette-Guérin, and Corynebacterium parvum. This form of immunotherapy has generally been proven ineffective and has mostly been abandoned [23].

The interferons were discovered in 1957 [70], and the first experience in the treatment of patients with mRCC was published in 1983 [71]. Interferons are glycoproteins produced by mammalian cells in response to viral infections or other inducers. Three major classes of interferons have been characterized, interferon-alpha (IFN-α), interferon-beta (IFN-β), and interferon-gamma (IFN-γ). IFNs can be produced in large quantities using recombinant DNA techniques. The interferons have a direct antiproliferative effect on renal tumor cells in vitro [72], stimulate host mononuclear immune cells, and enhance the expression of major-histocompatibility-complex molecules. IFN-α was the first active antitumor agent with reproducible activity in patients with mRCC. In total, there have been four randomized trials that compared a control of non-interferon therapy with IFN-α alone or in combination with a non-IFN-α therapy in mRCC [73-76]. The results of the two most important trials are displayed in Table 6. A Cochrane review of 42 randomized trials involving 4216 patients has confirmed the value of IFN-α in mRCC [77]. However, IFN-α as monotherapy provides only a modest/minimal survival benefit of median 2.6 months compared with controls and a reduction in 1-year mortality by 27% [77]. Only 3% of patients were alive at 5 or more years following IFN-monotherapy [78].

Interleukin-2 (IL-2) was described by Morgan et al [79] in 1976. IL-2 is a 15-kDa glycoprotein, naturally secreted by activated T lymphocytes, mostly $CD4^+$ cells. Recombinant IL-2 has been available since 1985. The molecular structure of IL-2 appears similar to that of granulocyte-macrophage colony-stimulating factor and IL-4 [80]. IL-2 mediates its biological effect by binding to the IL-2 receptor [19]. Thus, IL-2 has no direct impact on the tumor cells [81] but mediates antitumor activity through the modulation of the host's immune response [82].

In 1985, Rosenberg et al at the National Cancer Institute reported the first observations in human on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer [83]. This study established that immunological manipulations by the administration of IL-2 to patients with metastatic cancer may mediate a dramatic tumor regression. In 1995, Fyfe et al published the mature results obtained in 255 patients with mRCC treated with high-dose (HD) intravenous (iv) bolus single-agent IL-2 in seven clinical studies conducted at 21 institutions [84]. In this patient population, 15% had an objective response (CR: 7%; PR: 8%). Responses occurred in all sites of disease, including bone, intact primary tumors, and visceral metastases, and in patients with large tumor burdens or bulky individual lesions. The 5-year survival rate was 18% and the 10-year survival rate was 10% [85]. These patients are probably cured. These results led to the USA Food and Drug Administration (FDA) approval for the treatment of mRCC in 1992. Thus, IL-2 became the first biological agent to gain such approval for this disease. It should be noted that the Danish Medical Agency approved IL-2 for the treatment of mRCC in 1989.

However, toxicity related to HD bolus iv IL-2 is significant. Fyfe et al [84] reported fever, hypotension, oligouria with marked fluid retention, pulmonary edema, mental confusion, hepatic dysfunction and capillary leak syndrome. Totally, 4% of patients died of adverse events judged to be possibly or probably treatment-related and 15% of patients required admission to an intensive care unit.

With the intention to diminish the severe side effects, clinical trials evaluating continuous iv (civ) IL-2 [86] or subcutaneous (sc) IL-2 [87] were initiated. Low doses of IL-2 can be given by sc injection to outpatients, and have an acceptable risk of toxic effects for less physically fit patients [87]. At least 116 phase I-III trials of IL-2 based im-

Table 6. Randomized phase III trials evaluating different regimens of cytokines in mRCC.

Author	Treatment	Number pts	Response rate	Median surv (mos)	Time to PD (mos)	1-year survival	5-year survival
Gleave [50]	IFN-gamma	90	4.4%	12.2	1.9	50%	-
	Placebo	91	6.6%	15.7	1.9	50%	
MRC [75]	IFN-alpha	167	14%*	8.5	4.0*	43%*	-
	MPA	168	2%	6.0	3.0	31%	
Pyrhönen [76]	IFN-alpha + VLB	79	16.5%*	16.9**	3.2*	58%*	4%
	VLB	81	2.5%	9.5	2.2	38%	0%
Negrier [88, 91]	IL-2 iv +IFN-alpha	140	18.6%*	17	20%* §	-	10%
	Il-2 iv	138	6.5	12	15%		12%
	IFN-alpha	147	7.5%	13	12%		-
Yang [89]	IL-2, high dose iv	96	21%*	17	More durable	65%	20%
	IL-2, low dose iv	93	11%	17	CR's in high-	65%	15%
	IL-2, low dose sc	94	10%	17	dose group	65%	10%
McDermott [90]	IL-2, highdose iv	96	23.2*	17.5	3.1	-	20%
	IL-2, sc + IFN-alpha	96	9.9	13.0	3.1		18%
Negrier [92]	IL-2 sc + IFN-alpha	122	10.9%	15	-	-	-
	IL-2 sc	125	4.1%	15			
	IFN-alpha	122	4.4%	15			
	MPA	123	2.5%	15			

Abbreviations: *, significant difference; MPA, medroxyprogesterone acetate; VLB, vinblastin; §, data is 1-year progression-free survival; mos, months.

Table 7. Trials evaluating new biological "targeted therapies" in mRCC.

Author	Design	Number pts	Response rate	Median survival (months)	Time to PD (months)	1-year survival
Yang [113]	Bevacizumab, highdose	39	10%	No difference	4.8 (p<0.001)	No difference
	Bevacizumab, lowdose	37	0%		3.0	
	Placebo	40	0%		2.5	
Hainsworth [114]	Bevacizumab plus Erlotinib	63	25%	Not reached	11	78%
Motzer [115]	SU11248	63	40%	16.4	8.3	-
	(2 consecutive phase II)	106	40%	Too early	Too early	-
Rini	AG-013736	52	46%	Too early	Too early	Too early
ASCO abstract 4509						
Escudier	BAY 43-9006	Interim	Low but	Too early	6 (p<0.00001)	Too early
ASCO abstract 4510	Best supportive care	769	many SD		3	

Bevacizumab, Avastin®: monoclonal antibody against VEGF.
Erlotinib, Tarceva®: Tyrosin kinase inhibitor of EGFR.
SU11248, Sutent®: Tyrosin kinase inhibitor of PDGFR, VEGFR, c-KIT, FLT-3 [109].
AG-013736: Tyrosin kinase inhibitor of VEGFR1, VEGFR2, PDGFR.
BAY 43-9006, Sorafenib®, Tyrosin kinase inhibitor of CRAF, VEGFR2, VEGFR3, FLT-3, PDGFR, c-KIT [112].

munotherapy have been completed (reviewed in [82]). IL-2 has been used in different dose schedules applying various administration routes, as either monotherapy or in combination with other cytokines, chemotherapy, endocrine treatment and adoptive cellular immunotherapy. Although a large number of randomized trials have been performed with different treatment strategies, it still remains uncertain whether the dose or combination of IL-2 with other agents substantially influence treatment outcome [82].

The most important randomized phase III trials of IL-2 based immunotherapy are outlined in table 6. The combination of IL-2 and IFN-α resulted in a significantly higher response rate and an improved 1-year progression free survival, but did not increase overall survival [88].

Low-dose regimens have resulted in roughly equivalent response rates compared with high-dose regimens, but concerns have been raised as to whether the responses in the low-dose groups were as durable as those in the high-dose groups. Two randomized phase III trials, addressing this question, have been published recently [89, 90] (Table 6). Both studies reported a statistically significant increased response rate and a trend of more durable complete responses in favor of high-dose bolus iv IL-2 compared with low-dose sc IL-2. This difference did not, however, translate into an overall survival difference [89, 90] (Table 6).

A survival benefit of IL-2 has not been shown in randomized studies with a non-immunotherapy control [92, 93]. As seen from Table 6, all randomized trials have been small. No IL-2 treated group has exceeded 140 patients. This represents a too low statistical power to detect the long-term survival benefit actually seen in a small group of IL-2 treated patients. A large number of long-term follow-up reports have consistently been published demonstrating durable responses and long-term survival in a minority of patients (approximately 10%) treated with IL-2 [85, 91, 94, 95]. Thus, the true value of IL-2 lies in the probability that this drug can be curative for a small group of patients with metastatic disease [89]. The important issue is then to identify this subgroup of patients.

The initiation of the present thesis in 1999 represented the introduction in Denmark of IL-2 based immunotherapy as *home-based therapy*, in contrast to previous in-hospital treatment regimens [86, 96]. Due to the outpatient nature of the protocols, patients received instruction, guidance and monitoring during the first days of IFN-α/IL-2/histamine injections before self-administration in the patients home. Thereafter, only 2-3 ambulatory visits per months were scheduled within the following 3-9 months. A telephone "hot line" and skilled staff at the department of oncology in day-and-night preparedness is a prerequisite for the feasibility of this potentially toxic treatment – in patients with metastatic disease – on an outpatient setting.

TREATMENT OF METASTATIC RCC – OTHER THERAPIES

A variety of other biologic approaches to therapy have been investigated. No convincing benefit has been shown by using lymphokine-activated killer (LAK) cells [97], tumor-infiltrating lymphocytes (TIL) [98], dendritic cell vaccines [99], radioimmunotherapy with ^{131}I-labeled monoclonal antibody to G250 [100] and thalidomide [101, 102]. The value of adding cis-retinoid has been conflicting [103, 104]. Allogeneic stem cell transplant is promising, but deserves further study [105, 106].

However, within the last 2-3 years, based on increasing understanding of the biology associated with the von Hippel-Lindau (VHL) tumor suppressor gene inactivation, a large number (>20) of new drugs, "targeted biological therapies", have been assessed in mRCC and several of these have shown promising results [107-112]. The most important trials are shown in Table 7. All trials were investigating these new drugs as second-line after cytokine therapy. To date (March 2006), three studies have been regularly published [113-115]. At ASCO 2004, response rates of 40% were reported for the first time in mRCC [115]. At ASCO 2005 (Table 7), several studies confirmed these high response rates. Whether these will translate into improved survival rates is awaited. New trials have been initiated evaluating these new drugs as first line therapy, in combination with IL-2 and/or IFN-α and as adjuvant therapy.

IMMUNOLOGIC MECHANISMS OF ANTITUMOR ACTIVITY

20 years have passed since the first administration of IL-2 to cancer patients. During these years, reproducible dramatic durable tumor regressions have been noted after systemic administration of IL-2 in patients with mRCC. However, the exact mechanisms mediating tumor regression have not been identified. Whereas IL-2 has no direct impact on tumor cells, which can grow unimpeded in vitro [81], it has been assumed that the impact of IL-2 on tumors in vivo derives from its ability to expand and activate lymphocytes with anti-tumor activity [81]. In fact, IL-2 has effects on several immune cells including T-cells [116], natural killer (NK) cells [117, 118], B-cells [119], monocytes/macrophages [120] and neutrophils [121] by binding to subunits of the IL-2 receptor on these cells. Three different IL-2 receptor complexes exist consisting of various subunits; (i) the α-chain (CD25); (ii) the β-chain (CD122); and (iii) the γ-chain (CD132) [122]. In addition, other immune cells are activated by cytokines produced by activated T-cells and NK cells, such as interferon-γ, granulocyte-macrophage colony stimulating factor, and tumor necrosis factor-α. The biologic effects of IL-2 has been reviewed [19] however, with no clear consistent finding [123, 124]. Thus, there is an essential need to explain which immune cell subset(s) is implicated in mediating tumor regression and to identify consistent markers of an immune response that correlate with clinical responses and overall survival.

Tumor immunology is a field with more questions than answers, i.e., is the glass half full [125] or half empty? A fundamental question is whether the immune system sees tumors as foreign or self [126]. A milestone in tumor immunology was the cloning in 1991 of

MAGE-1, the first tumor antigen recognized by cytolytic T lymphocytes [127]. However, most human antigens expressed on tumor cells are normal non-mutated differentiation antigens [128]. Antigens that are only expressed in association with cancer are rare, including RCC [129]. To date, only few tumor antigens have been identified [130], with the total of 8 in RCC: Adipophilin [131], Fibroblast growth factor-5 (FGF-5) [132], G250/CAIX [133], intestinal carboxyl esterase gene (iCE) [134], M-CSF [135], RAGE1, PRAME, and glycoprotein 75 [136], with the role of G250/CAIX most elucidated [137, 138]. However, the immunologic mechanisms of antitumor activity are not completely understood [139]. Until now, no correlation between clinical benefit and the detection of antigen-specific T-cell responses has been achieved [140]. Therefore, NK cells are particularly attractive for the treatment of cancer because, unlike T lymphocytes, their anti-tumor activity does not depend on recognition of tumor-specific antigens, which remain poorly defined for the vast majority of malignancies [118].

As most patients with metastatic disease fail to respond to immunotherapy, effective immunotherapy against cancer has been suggested to be a question of overcoming immune suppression and immune escape [141]. Escape from immune recognition may be achieved by three distinct but closely related principles (Table 8) (I). Escape by loss of tumor recognition (i.e., loss or alteration of molecules which are important for the recognition by, and activation of, the immune system) (II). Escape by loss of susceptibility (i.e., escape from the effector mechanisms of cytotoxic lymphocytes) (III). Escape by induction of immune suppression (i.e., induction of immune dysfunction) [142].

The present thesis has tested the hypothesis that effective immunotherapy against cancer is a question of overcoming immune suppression in terms of chronic inflammation and oxidative stress.

In basic tumor immunology, the negative effect of NO and H_2O_2, produced from activated macrophages and neutrophils, on T and NK cell functions is well-established [143]. Among macrophage and neutrophil products, reactive oxygen species (ROS) may not only induce genomic instability [144] and thereby potentially contribute to malignancy [145], but also damage anti-tumor immune effector cells [143, 146-149]. Intratumoral macrophages isolated from melanoma metastases inhibit NK cell function by the release of ROS [150]. Intratumoral NK and T cells isolated from mRCC show signs of oxidative damage [151, 152]. IL-2 cannot activate NK cells in vitro in the presence of monocytes or macrophages [153]. Taken together, H_2O_2 secretion by activated macrophages and neutrophils is one important mechanism behind the tumor-induced immune suppression with decreased signal transduction and poor effector functions of T and NK cells observed in cancer patients [143]. Monocytes/macrophages and neutrophils therefore represent an important drug-target for cancer treatment, with the aim of reducing the number and/or function of these cells. However, the *clinical impact* of this oxidative stress hypothesis in relation to IL-2 based immunotherapy was unknown at the initiation of the present thesis.

Table 8. Tree principles of immune escape mechanisms. Adopted from Malberg [142].

Principle	Mechanism
Escape by loss of tumor recognition	Loss of HLA-class I Defect antigen processing and presentation Loss of tumor antigen expression Shedding of MHC class I-related-chain molecules Downmodulation of activating receptors, including NKG2D and natural cytotoxicity receptors Expression of ligands for inhibitory receptors HLA-G, HLA-E, CD48) Counterattack (FasL, B7-H1, soluble HLA class I) Suppression by regulatory T cells ($CD4^+CD25^+$)
Escape by loss of susceptibility	Expression of FLICE-inhibitory protein Expression and shedding of decoy death receptors Dysfunctional signaling through death receptors Expression of inhibitors of apoptosis protein family members (surviving) Resistance to perforin by expression of protease inhibitor -9, Cathepsin B Alteration in the p53 pathway Overexpression of bcl-2
Escape by induction of immune suppression	Myeloid suppressor cells Secretion of immune suppressive cytokines (TGF-α, IL-10) Oxidative stress and production of free radicals Chronic inflammation

Extensive laboratory work for a decennium has identified histamine dihydrochloride (HDC) as an anti-phagocyte drug-candidate [154]. Targeting NADPH-oxidase through binding to the H_2-receptor on monocytes [155] and neutrophils [156], HDC specifically blocks the formation and release of hydrogen peroxide (H_2O_2), thereby protecting NK and T cells from oxygen radical-induced inhibition and apoptosis [148]. Thus, NK and T cells remain viable and responsive to IL-2 [153, 157, 158].

In the present thesis, we have clinically explored this potential mechanism in two phase II trials in mRCC in collaboration with oncological departments in Sweden and United Kingdom, representing the introduction of histamine in patients with mRCC. As a supplement to the clinical trials, we initiated biological studies based on serial blood and tumor samples to search for a potential histamine effect in situ.

PATIENT SELECTION

Efforts to improve overall results also include the identification of prognostic factors, which allow treatment to be better directed towards those patients most likely to benefit. Given the toxicity and cost of IL-2 therapy, the benefit of a model predicting response as well as long-term survival to such therapy would represent a significant advance.

AIMS OF THE STUDIES

Two critical questions have driven the present thesis. First, which properties of the immune system are responsible for the dramatic tumor regression seen in some patients with mRCC following IL-2 administration? And second, can histamine increase the efficacy of IL-2 based immunotherapy by ending the immune suppression induced by phagocyte generation of reactive oxygen species, i.e. does a *clinical* testing of histamine support the oxidative stress hypothesis formulated in a pre-clinical setting?

The aims of this thesis were as follows:

- To improve the treatment of patients with mRCC.
- To identify parameters that may help to identify patients more likely to benefit from IL-2 based immunotherapy, either at baseline or as early as possible within the treatment course.
- To increase our understanding of the immunologic mechanisms of IL-2 based immunotherapy.

PATIENTS AND METHODS

In the following, an overall overview of the patients, treatments, number of blood- and tumor samples and applied methods is presented. A more detailed description is given in the individual articles.

PATIENTS

All data of this thesis were prospectively collected. A total of 181 patients, encompassing 120 Danish patients, 20 Swedish patients and 41 patients from United Kingdom entered phase II clinical trials (n=150) or were treated outside protocols as standard therapy (n=31). No patients were lost to follow up. Only Danish patients were asked for informed consent to the supplemental blood and tumor samples (Figure 2).

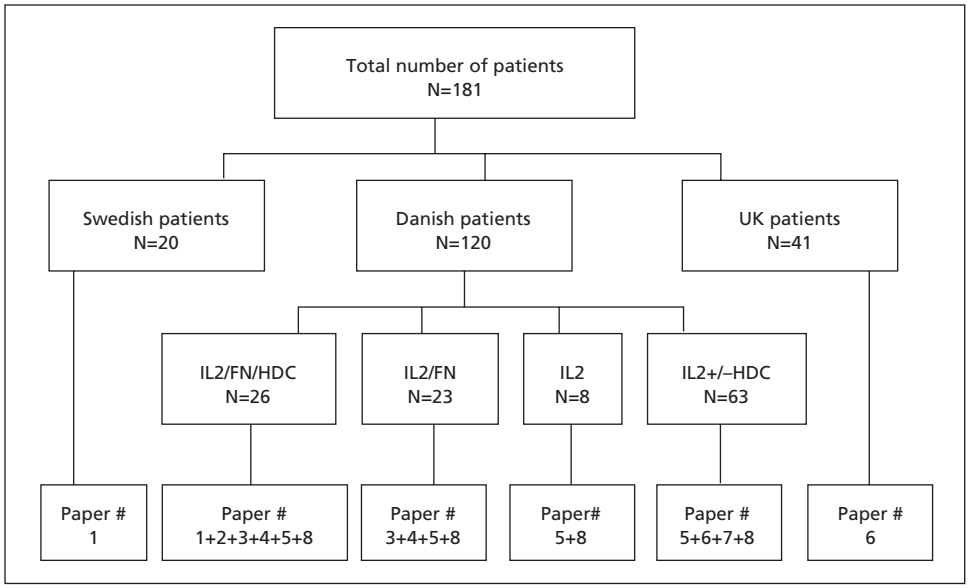


Figure 2. The total number of patients and their inclusion into article number 1-8.

ETHICS

Approval from the Ethics committees and the Medical Agencies in Denmark, Sweden and UK were obtained before start of the studies. Written informed consent was obtained from all patients before treatment initiation and before blood- and tumor biopsy sampling.

TREATMENTS

All patients received s.c. IL-2 based immunotherapy at low or intermediate dose levels. Due to the outpatient nature of the treatment, patients received instruction, guidance and monitoring during the first days of injections before self-administration in the patients home. Thereafter, only 2-3 ambulatory visits per months were scheduled within the treatment course. An overview of the protocols is given in Table 9 and a graphical overview of the schedules is given in Figure 3.

Main inclusion criteria were bidimensionally measurable, histologically confirmed mRCC; progressive disease; age 18 to 75 years; Karnofsky performance status ≥70; no brain metastases. Pre-study, all patients had a brain-, chest-, abdominal- and pelvic CT scan plus a bone scan. Bone lesions on bone scans were verified by CT scans or X-ray.

CRITERIA FOR TUMOR RESPONSE

Patients were evaluated for objective response, according to standard WHO criteria [159], (a) complete response (CR), defined as total disappearance of all clinical disease; (b) partial response (PR), defined as a reduction of more than 50% in the bidimensionally product diameter; (c) stable disease (SD), defined as a reduction of less than 50% or an increase in size of less than 25%; and (d) progressive disease (PD), defined as an increase in size of more than 25% in the bidimensionally product diameter or appearance of new lesions.

BLOOD SAMPLES

At baseline, all patients had a blood sample, which included differential count, hemoglobin, platelet count, creatinine, sodium, potassium, glucose, bilirubin, alkaline phosphatase, albumin, ALAT, LDH, calcium and ion-calcium and partial thromboplastin time (APTT).

For flow cytometry and cytotoxicity assay, a total of 250 blood samples were obtained serially during treatment in paper II. An additional 28 and 165 blood samples were obtained for the analysis in paper III and VII, respectively. Thus, a total of 443 blood samples were analysed by flow cytometry and cytotoxicity assays.

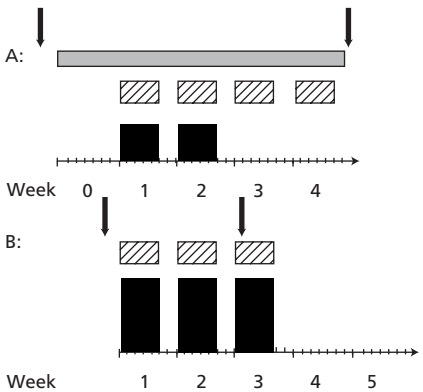
TUMOR SAMPLES

Core needle biopsies (18G cutting needle) were collected by standard ultrasound-guided procedures [160]. The tissue processing was standardized. Thus, all patients had baseline biopsies and repeated biopsies at day 2 in week 3 and week 8 (non-IFN containing regimen) or at day 1 in weeks 5, 12, 19, 24, 31 and 36, if possible (IFN-containing regimen), (Figure 3 and Table 10 and Table 11). The time-points were selected to coincide with routine outpatient visit, according to the immunotherapy schedule. The biopsies were obtained within the first days of the week, allowing almost identical fixation time for the tumor tissue.

Of the 120 consecutive Danish patients, written informed consent and baseline biopsies were obtained from 101 patients. Four patients did not complete one course of therapy because of toxicity and were not evaluable for objective response. Two patients had only fine needle biopsies performed. These 6 patients were excluded

Table 9. Overview of treatments.

Dose level	Treatment	N
Low-dose (Fig 3A)	IL-2 + IFN-α + histamine	26 + 20 Swedish patients
Low-dose (Fig 3A)	IL-2 + IFN-α	23
Intermediate-dose (Fig 3B)	IL-2 +/- histamine	63 + 41 UK patients
Intermediate-dose (Fig 3B)	IL-2	8



A: ▫ IFN-α, 3 MIU × 1 sc, ■ IL-2, 2.4 MIU/m2 × 2 sc, ▨ Histamine 1 mg × 2 sc, One cycle = 4 weeks, max 9 cycles
B: ■ IL-2, 18 MIU sc × 1, ▨ Histamine 1 mg × 2 sc, 2 weeks rest, One cycle = 5 weeks, max 4 cycles. Arrows indicate tumor biopsy time points

Figure 3. Treatment schedule.

Table 10. Number of patients having a biopsy.

Dose level	Week						
Low dose	w0	w5	w12	w19	w24	w31	w36
	38	29	24	12	9	3	4
Intermed.	w0	w3	w8	w13	w18		
	57	40	28	5	3		
Total	95	69					

Timing of tumor biopsies is illustrated in Fig 3.
Week 3 and week 5 biopsies were classified as "on-treatment biopsies" in paper # 5.

Table 11. Number of patients with a sufficient biopsy

Dose level	Week						
Low dose	w0	w5	w12	w19	w24	w31	w36
	34	25	13	7	6	1	2
Intermed.	w0	w3	w8	w13	w18		
	52	33	26	5	3		
Total	86	58					

Timing of tumor biopsies is illustrated in Fig 3.
Week 3 and week 5 biopsies were classified as "on-treatment biopsies" in paper # 5.

from the tumor analyses. Thus, 95 patients had baseline assessable tumor biopsies. The total number of patients having a tumor biopsy is outlined in Table 10. Eleven patients had more than one biopsy obtained at each time point, most frequently from both the primary kidney tumor and a metastatic lesion.

Carefully evaluation of the biopsies demonstrated insufficient tumor tissue and necrosis in 59 biopsies (21%) of a total of 284 biopsies obtained, giving 225 sufficient tumor biopsies. The number of patients with a sufficient tumor biopsy for immune and tumor cell evaluation is indicated in Table 11.

Two cases of deviations from Table 11 and paper V and VIII should be mentioned. In paper V, tumor FasL staining could not be assessed on week 5 in one patient treated with low-dose IL-2, IFN-α and histamine, rendering a total of 57 patients for the analysis on-treatment in paper V. This patient had, however, detectable intratumoral immune cells as well as KI-67-positive tumor cells, and therefore this patient was included in the analysis in paper II, III and IV.

In one patient with a baseline biopsy only, the intratumoral $CD57^+$ lymphocytes could not be assessed as the neoplastic cells also stained positively for this marker. In this patient, all other immune staining was successful and, thus, this patient was not excluded from the analyses in paper II, III, IV and V. However, for paper VIII, only patients with a complete set of data were included in the multivariate analysis, rendering a total of 85 patients at baseline for this paper.

Tumor specimens were classified by histological subtype and Fuhrman nuclear grade by a single central pathologist.

METHODS – IMMUNOHISTOCHEMISTRY

Since the introduction in 1991 of microwave-oven antigen retrieval in formalin-fixed, paraffin-embedded tissues as an enhancement method for immunohistochemical staining [161], immunohistochemistry of formalin-fixed, paraffin-embedded tissue has been increasingly used and has been thoroughly tested over years [162]. By using this technique, it is possible – in situ – to localize immune cells within the tumor nests and to locate the protein in cell type and cell structure (nucleus, cytoplasm, membranes). The superior morphology, easy storage for reuse, as well as the feasibility of cutting numerous 2 μm sections from a small core biopsy (measuring at a maximum 1 mm in diameter and 1 centimeter in length) favors paraffin-embedding technique compared with frozen-tissue. However, the precise concatenation of methodological details has a major effect of the final results and, thus, standardization of all procedures is important [162]. Within the present thesis, we focused on (i) standardization of the length of time for the formalin fixation and paraffin embedding; (ii) the time span between cutting of sections and staining as short as possible (few weeks) to avoid protein degradation; (iii) standardization for the microwave oven heating process, e.g. constant target retrieval buffer volume, constant processing time, constant number of slides, – and the use of a microwave oven with a turntable; (iv) standardization of procedures for immunohistochemistry staining. Only well-known and commercially available antibodies were used. All immunostaining were performed in an automate DAKO TechMate staining machine with a capacity of 120 slides per run. We used EnVision [163] as a simple, two-step visualization system of very high sensitivity to avoid problems with endogenous biotin in tissues as heat-induced epitope retrieval procedures may enhance the reactivity of endogenous biotin [162] highly expressed in especially kidney and liver tissues. Overall, as a result of the standardization efforts, day-to-day variation was reduced and variation contributed by laboratory processing was minimized, but not deleted.

METHODS – ASSESSMENTS OF INTRATUMORAL IMMUNE CELLS

Immunohistochemistry studies normally use semiquantitive scoring assessment of intratumoral immune cells. Based on the observation that immune cells not necessarily infiltrate tumor areas in a homogeneous distribution, we used stereological examination in measuring intratumoral immune cells in order to avoid sampling bias [164]. Unfortunately, this technique is associated with considerable workload and time consumption.

Stereology is mostly about sampling and only little about estimation [165]. The sampling principles are simple: systematic, uniformly random sampling at all levels [165, 166]. The optical fractionator principle is the classical method [167]. Despite only a small fraction of the tumor specimen is actually needed for estimation, the method requires initially the whole tumor for unbiased serial sections. Ultimately, a fraction of the sections is sampled for the study. This final sample is cut by at least 25 μm after processing, drying and mounting, and is the subject for identifications of particles by microscopy [168]. Immunohistochemistry is not implemented in stereology because of the requirement of thick sections.

Special issues concerning stereological examination in the present thesis should be mentioned:

(i) As the assessments was based on core biopsies obtained from accessible tumors, the fractionator principle was not applicable [167], and the material is, thus, biased by definition. Thus, estimation the total number of immune cells within the whole tumor was not possible. Therefore we only estimated the number of immune cells/mm^2 tumor.

(ii) Immunohistochemistry was a prerequisite in the present study as identification of lymphocyte subsets based on morphology alone was impossible. Thus, we were unable to use the disector method. However, as lymphocytes are small and completely uniform cells, we assumed that thin sections might be sufficient for the estimates.

(iii) We only assessed one section of each immune staining per patient. This was done because of the workload and also because of the cost of immunohistochemistry. However, assessing more than one section would have reduced the contribution of variance at this level.

(iv) Whereas patients with progressive disease (PD) had very few and easily detectable intratumoral lymphocyte subsets, responding patients had large numbers of lymphocyte subsets, which in several cases made enumerations difficult. Moreover, whereas tumors in patients with PD remained unchanged, tumors in responding patients loosened, the tumor cell density per mm^2 tumor diminished, and fibrotic areas developed. This observation has also been described in regressing tumors in

metastatic melanoma [169]. According to our counting rules, necrotic and fibrotic areas were avoided and only a cell with staining restricted to the plasma membrane, a visible nucleus and located within the counting frame was counted as positive. The unit for intratumoral immune cell infiltration was given as number of immune cells per mm^2 tumor. This unit does not take into account regressive changes in responding patients. The immune – tumor cell ratio would have been a more accurate unit for the measurements. Thus, we may have underestimated the immune infiltrate in responding patients.

(v) Using this technique with an unbiased counting frame, a high level of reproducibility was found, as has also been demonstrated by others [170, 171].

METHODS – CHROMIUM-RELEASE ASSAYS

In order to assess the killing potential of NK cells – which should be regarded as the most important functional capability – the ^{51}Cr-release 4-hour assay against the NK sensitive target K562 cell line was used. This assay is the gold standard but is, however, associated with a considerable workload [172].

METHODS – FLOW CYTOMETRY

Flow cytometry employes instrumentation that scans single cells flowing past excitation sources in a liquid medium. The technology is unique in its ability to provide rapid, quantitative, multiparameter analyses on single cell characteristics [173]. Flow cytometry has been thoroughly tested over years. The Flow cytometer used in the present thesis was regularly calibrated. Lymphocytes were gated on the basis of forward and side scatter. At least 10^4 cells were analysed for each sample. The percentage of lymphocyte subsets obtained by flow cytometry was converted into absolute numbers by multiplying with the simultaneously obtained differential lymphocyte count.

METHODS – STATISTICS

The statistical methods are standard for this type of data. P-values are two sided. For comparing two unpaired groups, the nonparametric Mann-Whitney U-test or Fisher's exact test were used. For comparing two paired groups, the Wilcoxon signed rank test was used. For comparing three or more unmatched groups, the Kruskal-Wallis test was used. The cumulated survival rate was analysed by Kaplan-Meier and the log-rank test was used to analyse for survival differences among subgroups of patients. Overall survival was measured from first day of treatment until death or last follow-up evaluation. Time to progressive disease was measured from first day of treatment until disease progression. All calculations were performed using SPSS 10.0 and 11.0 statistical software.

RESULTS

An overall overview of the results that form the thesis is summarised in the following. A detailed description of the results is given in the individual articles.

1. The thesis represented the establishment in Denmark of s.c. IL-2 based immunotherapy for mRCC as an outpatient hometherapy and, moreover, documented the safety and feasibility of the treatment (Article I and VI).
2. Of 120 Danish patients with mRCC – initially estimated as noncandidates for curative surgery – receiving IL-2 based immunotherapy, a total of 12 patients (10%) achieved no evidence of disease (NED) after either immunotherapy-only (n=5), immunotherapy plus surgical resection of residual disease (n=5) or surgery plus re-treatment with immunotherapy (n=2).
3. Of 120 Danish patients receiving IL-2 based immunotherapy, the estimated 5-year survival rate was 16% (Paper VIII). This seems consistent with results previously obtained with intermediate to high doses of i.v. IL-2 based immunotherapy (Figure 4).
4. IL-2 based immunotherapy, administered subcutaneous in low- or intermediate dose levels, was able to induce objective tumor responses in a minority of patients (Paper I, III, VI and VIII) Figure 5.
5. The present thesis represented the introduction of histamine as an adjuvant to IL-2 based immunotherapy in mRCC. In a low-dose schedule of IL-2 and IFN-α, histamine did not appear to add efficacy (paper I). However, in two randomised phase II studies, in which the dose of IL-2 was doubled and IFN-α was omitted, the Danish study showed a trend towards benefit in favor of IL-2/histamine, whereas the UK study was negative for all end points (Paper VI). Thus, a randomised phase III trial is warranted to clarify the potential role of adding histamine to IL-2 in mRCC.
6. The data obtained from blood and tumor analyses provided novel in vivo evidence of the possible contribution of *lymphocyte subsets* – T cells and NK cells – in the tumor reduction in responding patients during IL-2 based immunotherapy (Paper II, III, V and VII).

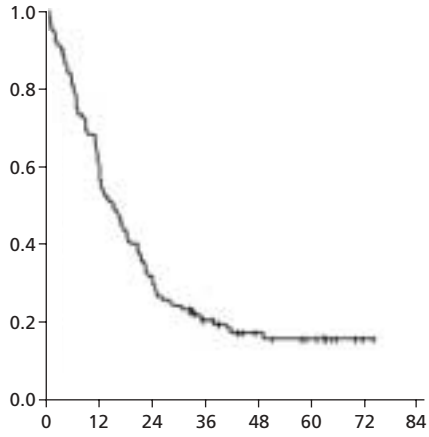


Figure 4. Overall survival of 120 patients with mRCC receiving IL-2 based immunotherapy. Median survival 15.1 months. Estimated 5-year survival 16%. Vertical and horizontal axes, the survival probability and months of follow-up, respectively. Tick marks (|) indicate last date of follow-up.

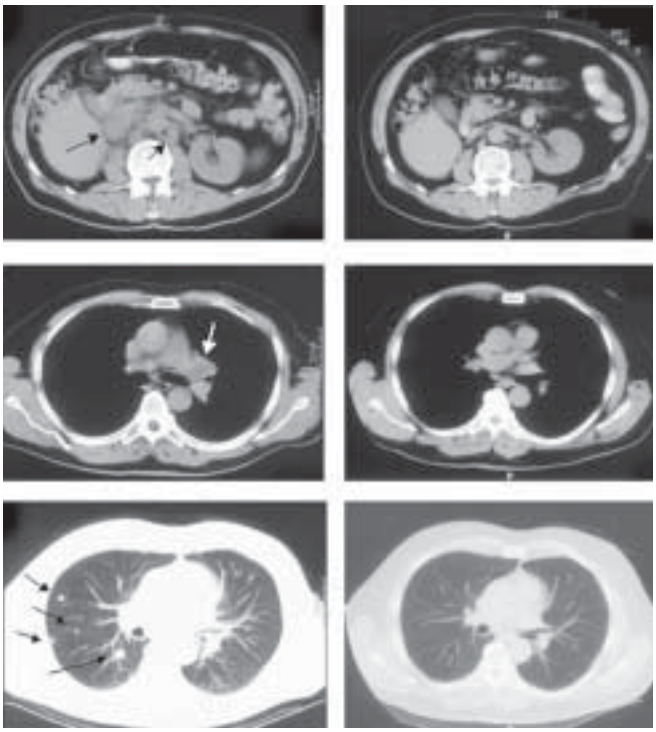


Figure 5. A patient with baseline lung metastasis and lymph node metastases in retroperitoneum and left hilus (left lane, arrows). The patient received IL-2 and histamine and achieved complete response. The patient is alive with no recurrence after 43 months of follow-up.

7. In responding patients, cytokine therapy induced substantial changes in the blood and tumor tissue leukocyte composition, correlated with both response and survival. In contrast, in progressing patients, both the absolute number and the relative composition of leukocyte subsets in blood and tumor tissue remained unaffected of cytokine therapy (Paper II, III and V).
8. Circulating as well as intratumoral *phagocytes* – monocytes and neutrophils – were shown to be powerful negative prognostic factors for IL-2 based immunotherapy (Paper III, VII and VIII).
9. The thesis emphasized the value of accompanying clinical trials with blood and tumor tissue assessments. Based on the randomised trials by themselves, no effect of adding histamine to IL-2 was recognized. However, by the biological analyses, a potential effect of histamine was clearly demonstrated (Paper VII).
10. Our data suggested that IFN-α in vivo had only modest effect on tumor proliferation in patients with mRCC. Tumor Ki-67 (MIB-1) reactivity after one month of immunotherapy appeared to be a significant predictor of patient survival (Paper IV).
11. Our observations did not support the hypothesis that FasL tumor "counterattack" has an effect on the clinical outcome in metastatic renal cell carcinoma during IL-2 based immunotherapy (Paper V).
12. The thesis pointed on five clinical and three supplemental immunological independent prognostic factors of survival in patients with mRCC receiving IL-2. The three independent immunological parameters had significant discriminatory power as supplemental risk factors in prognostic models based on the clinical risk factors, identifying subgroups within the favorable clinical group with estimated 5-year survival rates of 60%, 25% and 0%, respectively (Paper VIII).

DISCUSSION

Detailed discussions of the different aspects of the thesis are included in the individual articles. The main and central issues are further discussed in the following.

With a worldwide mortality of 102,000 in the year 2002, RCC remains a major challenge for the oncologist. There is no effective treatment for the large majority of patients where the disease is a much more powerful determinant of outcome than any therapy [77]. Thus, RCC remains a model for the translation of laboratory findings to the clinic and the treatment of patients with innovative therapeutic strategies. The present thesis adds to these efforts by evaluating the oxidative stress hypothesis in a clinical setting.

IL-2 was approved by the Danish Medical Agency in 1989 and by the US Food and Drug Administration in 1992 for the treatment of metastatic RCC. The principal justification for this approval was the ability of IL-2 to induce dramatic and durable responses in a minority of patients in this previous completely treatment-refractory disease. After 20 years, however, the value of immunotherapy for inoperable locally advanced and mRCC is still controversial. The variable natural history with even "spontaneous" remissions, the lack of randomized phase III trials evaluating IL-2 with a placebo control arm – and, probably, the high level of toxicity related to the drug – have nourished skepticism about IL-2 [77]. In fact, despite a yearly worldwide mortality of approximately 100,000, the large majority of patients are not offered active treatment and supportive care alone are considered standard of care in many centers [59]. The majority of the remaining patients are offered IFN-α despite its modest activity with a median survival improvement of 2.6 months and a reduction of the 1-year mortality by 27% [77]. A minority of patients receive sc IL-2 and only approximately 100 patients worldwide per year receive high-dose bolus iv IL-2. However, one should bear in mind that the US FDA as well as the Danish Medical Agency in fact *have* approved IL-2 for the treatment of mRCC. Furthermore, a large number of long-term follow-up reports – including the results from the present thesis – have consistently been published demonstrating durable responses and long-term survival in a minority of patients treated with IL-2 [85, 91, 94, 95]. Thus, the true value of IL-2 lies in the probability that this drug can be curative for a small group of patients with metastatic disease [89]. The important issue is then to identify this subgroup of patients. The present thesis suggests a prognostic model based on clinical factors supplemented with immunological factors that may help to select patients more likely to benefit from IL-2 based immunotherapy.

IL-2 works entirely through activation of the patient's endogenous immune system. However, the exact mechanism of action is unclear. Unraveling the mechanisms involved in tumor rejection is very important as this could result in more effective immunotherapy strategies against cancer. There are three requirements for an effective immunotherapy for cancer: (i) a sufficient number of tumor reactive immune cells must be present – or must be generated – in the tumor-bearing host; (ii) these immune cells must be capable of reaching the tumor, extravasating at the site of the cancer and infiltrating the tumor stroma; and (iii) the immune cells at the tumor site must have appropriate effector mechanisms to destroy cancer cells [128, 174]. The present thesis adds to our understanding of the immune cell orchestration in relation to IL-2 based immunotherapy and the correlation of these immune cell subsets with objective response and/or survival. However, only indirect evidence of a cellular immune response in responding patients is provided.

The present thesis represents the first systematic collection of serial tumor core biopsies and blood samples during IL-2 based immunotherapy in mRCC. For comparison, the study by Bukowsky [175] comprised 27 biopsies (13 at baseline and 14 during or after therapy) from 17 patients; the study by Cohen [176] included one patient (baseline and after therapy biopsy); and the study by Rubin [177] included 12 patients with baseline nephrectomy only. Only the study by Bukowsky [175] also included blood samples, but these were obtained at different time points than the tumor biopsies. No consistent finding has been demonstrated in these studies. Thus, an effort to integrate immune cellular infiltrates with standard clinical criteria for tumor regression has largely not been done [174]. For comparison, based on 443 serial blood samples and 225 serial tumor core biopsies in the present thesis, we were able to map the orchestration of immune cells in blood and tumor at baseline and during IL-2 based immunotherapy. An understanding of IL-2 based immunotherapy as a "targeted therapy" requiring immune cells for tumor rejection emerged from these analyses. Moreover, the "image" of the immune cell composition in peripheral blood seems "delayed" compared with the "image" of the intratumoral immune cell composition. In short, analyses revealed that high numbers of baseline intratumoral $CD4^+$, $CD8^+$, $CD56^+$ and $CD57^+$ T- and NK cells and high numbers of on-treatment intratumoral $CD3^+$, $CD4^+$, $CD8^+$ and $CD57^+$ T- and NK cells were significantly correlated with objective response. In contrast, no baseline blood immune cells were correlated with objective response. High numbers of on-treatment total blood lymphocytes, $CD3^+$, $CD3^+zeta^+$ and $CD57^+$ lymphocyte subsets and low numbers of blood neutrophils were significantly correlated with objective response. The immune cells were also correlated with survival. These analyses revealed that no blood lymphocyte subsets were correlated with survival whereas high numbers of baseline blood neutrophils, on-treatment blood neutrophils and on-treatment blood monocytes were correlated with short survival. Of intratumoral immune cells, high numbers of baseline $CD57^+$, baseline $CD4^+$, and on-treatment $CD3^+$ were significantly correlated with favorable survival whereas presence of baseline neutrophils was correlated with short survival. Thus, neutrophils and monocytes/macrophages are "bad guys" and T cells and NK-cells are "good guys" for the outcome of IL-2 based immunotherapy. All statistical differences for blood and tumor phagocytes have the same negative direction with both objective response and survival as endpoint. In contrast, all statistical differences for blood and tumor lymphocyte subsets have the same positive direction with both ob-

jective response and survival as end-point. Moreover, in responding patients we were able to demonstrate a systemic as well as a local recruitment of immune effector cells (i.e. T- and NK cells) whereas in progressing patients, the immune cells remained totally unaffected by IL-2 treatment. In patients with stable disease, the changes were in between. Our data were in line with findings in mRCC [19, 175-179] and malignant melanoma [177, 180, 181] and support the notion that IL-2 mediates antitumor activity through the activation of the host's immune response [82, 123]. The biological results obtained in the present thesis should prompt further research. Immunotherapy should always be accompanied by laboratory questions based on blood and tumor sample collections.

Histamine dihydrochloride as an adjunct to IL-2 is an example of translational research from basic biology to clinical trials. Following the first observation in 1982 by Seaman et al [146], a large number of in vitro and in vivo observations from independent laboratories have supported the observation of oxidative suppression of NK and T cells by phagocytes (i.e. monocytes, macrophages and neutrophils) [147, 150-152, 182]. Therefore, in basic tumor immunology – in the preclinical setting – the negative effect of NO and H_2O_2 produced from activated macrophages and neutrophils on T and NK cell functions is well-established [143]. The observations by Kristoffer Hellstrand et al that histamine protects NK and T cells against oxygen radical-induced dysfunction and apoptosis by specifically blocking the formation and release of hydrogen peroxide (H_2O_2) from phagocytes, and moreover, maintains the activation of NK and T cells by IL-2 and/or IFN-α, are compelling [148, 153, 155, 157, 158, 183-186]. Therefore, the pre-clinical basis for introducing histamine in combination with IL-2 and/or IFN-α in clinical trials was obvious. Despite that, several clinical trials of histamine in combination with IL-2 and/or IFNα were unsuccessful in acute myelogenous leukaemia [187], chronic hepatitis C [188], multiple myeloma [189], metastatic melanoma [190-192] and mRCC [193]. However, our biological analyses in mRCC (paper VII) support the oxidative stress hypothesis [143, 154, 194-196] and a potential effect of histamine was clearly demonstrated. This emphasizes the value of accompanying clinical trials with blood and tumor tissue assessments. Indeed, our assessment of the oxidative stress hypothesis in blood and tumor tissue is the first to establish a biological rationale – in human – for the use of histamine in conjunction with IL-2. Thus, targeting H_2O_2 by histamine seems to enhance the antitumor activity of IL-2 in situ in a subgroup of patients with low monocytes/neutrophils or high NK cells. The explanation for the lack of a histamine-effect when IFN-α was co-administered is unclear.

Since its introduction to the clinic in 1985 [83], IL-2 remains the only established immunotherapy approved by the US Food and Drug Administration for the treatment of mRCC. However, despite 20 years of clinical trials, no combination therapy has proved better than IL-2 treatment alone in terms of long-term survival [88, 90]. Thus, cancer immunotherapy is most dependent on the application of advances in knowledge of basic science and for its translation to the clinic. The present thesis indicates a direction for future clinical immunotherapy efforts.

GENERAL CONCLUSIONS

- Manipulating the immune system by IL-2 based immunotherapy may induce durable tumor regression in mRCC.
- Outpatient s.c. IL-2 based immunotherapy in mRCC is feasible and safe. The estimated 5-year survival rate of 16% seems consistent with results previously obtained with intermediate to high doses of i.v. IL-2 based immunotherapy.
- Histamine did not appear to add efficacy in a low-dose schedule of IL-2 and IFN-α. However, a potential effect of histamine was clearly demonstrated in the blood and tumor analyses in a randomised phase II trial, in which the dose of IL-2 was doubled and IFN-α was omitted. Thus, a large randomised phase III trial is warranted – appropriately stratified for monocytes and neutrophils in blood and tumor tissue – to clarify the potential role of adding histamine to IL-2 in mRCC.
- The data obtained from blood and tumor analyses provided novel in vivo evidence of the possible contribution of *lymphocyte subsets* – T cells and NK cells – in the tumor reduction in responding patients during IL-2 based immunotherapy. In contrast, circulating as well as intratumoral *phagocytes* – monocytes and neutrophils – were shown to be powerful negative prognostic factors for IL-2 based immunotherapy.
- Tumor analyses revealed that high numbers of baseline intratumoral $CD4^+$, $CD8^+$, $CD56^+$ and $CD57^+$ T- and NK cells and high numbers of on-treatment intratumoral $CD3^+$, $CD4^+$, $CD8^+$ and $CD57^+$ T- and NK cells were significantly correlated with objective response.
- No baseline blood immune cells were correlated with objective response. High numbers of on-treatment total blood lymphocytes, $CD3^+$, $CD3^+zeta^+$ and $CD57^+$ lymphocyte subsets and low numbers of blood neutrophils were significantly correlated with objective response.
- No blood lymphocyte subsets were correlated with survival whereas high numbers of baseline blood neutrophils, on-treatment blood neutrophils and on-treatment blood monocytes were correlated with short survival.
- Of intratumoral immune cells, high numbers of baseline $CD57^+$, baseline $CD4^+$, and on-treatment $CD3^+$ were significantly correlated with favorable survival whereas presence of baseline neutrophils was correlated with short survival.
- In progressing patients, both the absolute number and the relative composition of leukocyte subsets in blood and tumor tissue remained unaffected by cytokine therapy. In contrast, cytokine therapy induced substantial changes in the blood and tumor tissue leukocyte composition in responding patients, correlated with both response and survival.
- Our data suggest that IFN-α in vivo had only modest effect on tumor proliferation in patients with mRCC. Tumor Ki-67 (MIB-1) reactivity after one month of immunotherapy appeared to be a significant predictor of survival.
- Our observations did not support the hypothesis that FasL tumor "counterattack" has an effect on the clinical outcome in mRCC during IL-2 based immunotherapy.
- The thesis pointed on five clinical (PS, bone metastases, lymph node metastases, low hemoglobin and high LDH) and three supplemental immunological (intratumoral $CD57^+$ NK cells <50 cells/mm^2, intratumoral neutrophils >0 and blood neutrophils >6.0) as independent prognostic factors of survival in patients with mRCC receiving IL-2. The three independent immunological parameters had significant discriminatory power as supplemental risk factors in prognostic models based on the clinical risk factors, identifying subgroups within the favorable clinical group with estimated 5-year survival rates of 60%, 25% and 0%, respectively.
- Immunotherapy should always be accompanied by laboratory questions based on blood and tumor sample collections.

FUTURE ASPECTS

The results obtained in this thesis have to be confirmed in independent and larger studies. First of all, the validation of our prognostic model – based on clinical factors supplemented with immunological factors – is of high priority as these features may help to select patients more likely to benefit from IL-2 based immunotherapy. A validation study is currently underway examining 120 patients treated at the Department of Oncology in Aarhus, between September 2002 and September 2004 with low-dose IL-2 and IFN-α (same schedule as in the present thesis). Moreover, an international kidney cancer working group has been established to identify independent, validated predictors of survival and ultimately to develop a single validated prognostic model, based on >4000 mRCC patients

receiving IL-2 based immunotherapy [197]. The possibility of validation of our three independent immunological features – blood neutrophils, tumor neutrophils and tumor $CD57^+$ NK cells – in this >4000 patient material will be pursued.

Secondly, the translation of our results from metastatic disease to primary RCC will be an important future aspect. We will evaluate our hypothesis – that IL-2 based immunotherapy is a "targeted therapy" requiring immune effector cells for tumorlysis – in an adjuvant setting. Thus, adjuvant IL-2 based immunotherapy should only be offered to patients with >50 $CD57^+$ NK cells/mm^2 tumor tissue, no intratumoral neutrophils, and blood neutrophils $< 6.0 \times 10^9$/L. This should be evaluated in a randomised adjuvant trial.

Third, future developing and assessing drugs that block the generation of oxygen radicals as an adjunct to IL-2 is a viable therapeutic opportunity in renal cell cancer. These drugs – including histamine – should be evaluated in randomised trials appropriately stratified for monocytes and neutrophils in blood and tumor.

Fourth, the improved understanding of the biology associated with renal cancer have generated a large number (>20) of new drugs, "targeted biological therapies", currently assessed in mRCC. Whether these new drugs – either alone, as first line therapy or in combination with IL-2 and/or IFNα – will improve survival rates will be the cutting edge questions for clinical trials in the foreseeable future. A lesson learned from the present thesis is that it would be beneficial to require (serial) blood and tumor tissue collections in parallel with these clinical studies.

SUMMARY

The present thesis consists of 8 published articles focusing on interleukin-2 based immunotherapy in metastatic renal cell carcinoma (mRCC). This disease represents a significant challenge, as the tumor is resistant to current chemotherapy, hormonal therapy and radiation therapy. However, IL-2 based immunotherapy may induce dramatic durable tumor regression by manipulating the immune system, however, only in a minority of patients.

Two critical questions have driven the present thesis. First, which properties of the immune system are responsible for the dramatic tumor regression seen in some patients with mRCC following IL-2 administration? And second, can histamine increase the efficacy of IL-2 based immunotherapy by ending the immune suppression induced by phagocyte-generation of reactive oxygen species?

120 Danish patients, 41 UK patients and 20 Swedish patients were treated with low- or intermediate dose IL-2 based immunotherapy in an outpatient setting. As monitoring of the Danish patients, 443 serial blood samples and 225 serial tumor core biopsies were obtained.

The regimen of outpatient *low-dose* subcutaneous IL-2 and IFN-α in mRCC is safe and active. In the Danish patients, an estimated 5-year survival rate of 16% was observed.

From the blood and tumor analysis, an understanding emerged that IL-2 based immunotherapy is a "targeted therapy" requiring intratumoral immune cells ($CD4^+$, $CD8^+$, $CD56^+$, $CD57^+$ T- and NK cells) for treatment effect. In contrast, monocytes and neutrophils were harmful for the outcome of IL-2 based immunotherapy. In progressing patients, the leukocyte subsets in blood and tumor tissue remained unaffected by cytokine therapy.

The fate of a patient with mRCC prior to IL-2 and IFN-α based immunotherapy cannot be determined by measuring baseline tumor features of FasL expression or Ki-67 (MIB-1) proliferation marker.

We established a biological rationale for the potential use of histamine in conjunction with IL-2 in mRCC. A large confirmatory randomised phase III trial of IL-2 with and without histamine in mRCC appropriately stratified for monocytes and neutrophils in blood and tumor tissue is warranted.

In a multivariate analysis, 5 clinical features (PS, bone metastases, lymph node metastases, low hemoglobin and high LDH) plus 3 supplemental immunological factors (intratumoral $CD57^+$ NK cells <50 cells/mm^2, intratumoral neutrophils >0 and blood neutrophils >6.0) were independent prognostic factors of short survival in patients with mRCC receiving IL-2, identifying subgroups with estimated 5-year survival rates of 60%, 25% and 0%, respectively. These features may help to select patients more likely to benefit from IL-2 based immunotherapy.

ABBREVIATIONS

CR:	Complete response
RCC:	Renal cell carcinoma
mRCC:	Metastatic rena cell carcinoma
HDC:	Histamine dihydrochloride
HLA:	Human leukocyte antigen
H_2O_2:	Hyrogen peroxide
IFN-*a*:	Interferon-alpha
IL-2:	Interleukin-2
LDH:	Lactate dehydrogenase
NK:	Natural killer cells
NO:	Nitrogen oxide
PR:	Partial response
PS:	Performance status
ROS:	Reactive oxygen species
SD:	Stable disease
PD:	Progressive disease
VHL:	von Hippel Lindau

THIS DOCTORAL THESIS IS BASED ON THE FOLLOWING ORIGINAL PAPERS

I. Donskov F, von der Maase H, Henriksson R, Stierner U, Wersäl P, Nellemann H, Hellstrand K, Engman K and Naredi P: Outpatient treatment with subcutaneous histamine dihydrochloride in combination with interleukin-2 and interferon-alfa in patients with metastatic renal cell carcinoma: results of an open, single-armed, multi-center phase-II study. Annals of Oncology 2002, 13: 441-449.

II. Donskov F, Bennedsgaard KM, von der Maase H, Marcussen N, Fisker R, Jensen JJ, Naredi P and Hokland M: Intratumoral and peripheral blood lymphocyte subsets in patients with metastatic renal cell carcinoma undergoing interleukin-2 based immunotherapy: association to objective response and survival. British J Cancer 2002, 87: 194-201.

III. Donskov F, Bennedsgaard KM, Hokland M, Marcussen N, Fisker R, Madsen HT, Fode K and von der Maase H: Leukocyte orchestration in blood and tumour tissue following interleukin-2 based immunotherapy in metastatic renal cell carcinoma. Cancer Immunol Immunotherapy 2004, 53: 729-739.

IV. Donskov F, Marcussen N, Hokland M, Fisker R, Madsen HT and von der Maase H: In vivo assessment of the antiproliferative properties of interferon-a during immunotherapy: Ki-67 (MIB-1) in patients with metastatic renal cell carcinoma (mRCC). British J Cancer 2004, 90: 626-631.

V. Donskov F, von der Maase H, Marcussen N, Hamilton-Dutoit S, Madsen HT, Jensen JJ and Hokland M: FasL expression in metastatic renal cell carcinoma during interleukin-2 based immunotherapy: No in vivo effect of FasL tumor counterattack. Clinical Cancer Research 2004, 10: 7911-7916.

VI. Donskov F, Middleton M, Fode K, Meldgaard P, Mansoor W, Lawrance J, Thatcher N, Nellemann H and von der Maase H: Two randomised phase II trials of subcutaneous interleukin-2 and histamine dihydrochloride in patients with metastatic renal cell carcinoma. British J Cancer 2005, 93: 757-762 .

VII. Donskov F, Hokland M, Marcussen N, Madsen HT and von der Maase H: Monocytes and neutrophils as "bad guys" for the outcome of interleukin-2 with and without histamine in metastatic renal cell carcinoma – results from a randomised phase II trial. British J Cancer 2006, 94, 218-226.

VIII. Donskov F and von der Maase H: Impact of immune parameters on long-term survival in metastatic renal cell carcinoma. Journal of Clinical Oncology, 2006, 24: 1997-2005.

REFERENCES

1. Parkin, D. M., Bray, F., Ferlay, J., and Pisani, P. Global cancer statistics, 2002. CA Cancer J Clin., 55: 74-108, 2005.
2. Lindblad, P. Epidemiology of renal cell carcinoma. Scand.J.Surg., 93: 88-96, 2004.
3. Mathew, A., Devesa, S. S., Fraumeni, J. F., Jr., and Chow, W. H. Global increases in kidney cancer incidence, 1973-1992. Eur.J.Cancer Prev., 11: 171-178, 2002.
4. Martel, C. L. and Lara, P. N. Renal cell carcinoma: current status and future directions. Crit Rev.Oncol.Hematol., 45: 177-190, 2003.
5. McLaughlin, J. K. and Lipworth, L. Epidemiologic aspects of renal cell cancer. Semin.Oncol., 27: 115-123, 2000.
6. Storkel, S., Eble, J. N., Adlakha, K., Amin, M., Blute, M. L., Bostwick, D. G., Darson, M., Delahunt, B., and Iczkowski, K. Classification of renal cell carcinoma: Workgroup No. 1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer, 80: 987-989, 1997.
7. Reuter, V. E. and Presti, J. C., Jr. Contemporary approach to the classification of renal epithelial tumors. Semin.Oncol., 27: 124-137, 2000.
8. Cohen, H. T. and McGovern, F. J. Renal-cell carcinoma. N.Engl.J Med., 353: 2477-2490, 2005.
9. Hemminki, K., Li, X., and Czene, K. Familial risk of urological cancers: data for clinical counseling. World J.Urol., 21: 377-381, 2004.
10. Linehan, W. M., Vasselli, J., Srinivasan, R., Walther, M. M., Merino, M., Choyke, P., Vocke, C., Schmidt, L., Isaacs, J. S., Glenn, G., Toro, J., Zbar, B., Bottaro, D., and Neckers, L. Genetic basis of cancer of the kidney: disease-specific approaches to therapy. Clin.Cancer Res., 10: 6282S-6289S, 2004.
11. Latif, F., Tory, K., Gnarra, J., Yao, M., Duh, F. M., Orcutt, M. L., Stackhouse, T., Kuzmin, I., Modi, W., Geil, L., and . Identification of the von Hippel-Lindau disease tumor suppressor gene. Science, 260: 1317-1320, 1993.
12. Schmidt, L., Duh, F. M., Chen, F., Kishida, T., Glenn, G., Choyke, P., Scherer, S. W., Zhuang, Z., Lubensky, I., Dean, M., Allikmets, R., Chidambaram, A., Bergerheim, U. R., Feltis, J. T., Casadevall, C., Zamarron, A., Bernues, M., Richard, S., Lips, C. J., Walther, M. M., Tsui, L. C., Geil, L., Orcutt, M. L., Stackhouse, T., Lipan, J., Slife, L., Brauch, H., Decker, J., Niehans, G., Hughson, M. D., Moch, H., Storkel, S., Lerman, M. I., Linehan, W. M., and Zbar, B. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat.Genet., 16: 68-73, 1997.
13. Tomlinson, I. P., Alam, N. A., Rowan, A. J., Barclay, E., Jaeger, E. E., Kelsell, D., Leigh, I., Gorman, P., Lamlum, H., Rahman, S., Roylance, R. R., Olpin, S., Bevan, S., Barker, K., Hearle, N., Houlston, R. S., Kiuru, M., Lehtonen, R., Karhu, A., Vilkki, S., Laiho, P., Eklund, C., Vierimaa, O., Aittomaki, K., Hietala, M., Sistonen, P., Paetau, A., Salovaara, R., Herva, R., Launonen, V., and Aaltonen, L. A. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat.Genet., 30: 406-410, 2002.
14. Nickerson, M. L., Warren, M. B., Toro, J. R., Matrosova, V., Glenn, G., Turner, M. L., Duray, P., Merino, M., Choyke, P., Pavlovich, C. P., Sharma, N., Walther, M., Munroe, D., Hill, R., Maher, E., Greenberg, C., Lerman, M. I., Linehan, W. M., Zbar, B., and Schmidt, L. S. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. Cancer Cell, 2: 157-164, 2002.
15. Atkins, M. B., Avigan, D. E., Bukowski, R. M., Childs, R. W., Dutcher, J. P., Eisen, T. G., Figlin, R. A., Finke, J. H., Flanigan, R. C., George, D. J., Goldberg, S. N., Gordon, M. S., Iliopoulos, O., Kaelin, W. G., Jr., Linehan, W. M., Lipton, A., Motzer, R. J., Novick, A. C., Stadler, W. M., Teh, B. T., Yang, J. C., and King, L. Innovations and challenges in renal cancer: consensus statement from the first international conference. Clin.Cancer Res., 10: 6277S-6281S, 2004.
16. Kaelin, W. G., Jr. The von Hippel-Lindau tumor suppressor gene and kidney cancer. Clin.Cancer Res., 10: 6290S-6295S, 2004.
17. Goldstein, N. S. The current state of renal cell carcinoma grading. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer, 80: 977-980, 1997.
18. Fuhrman, S. A., Lasky, L. C., and Limas, C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am.J.Surg.Pathol., 6: 655-663, 1982.
19. Bukowski, R. M. Natural history and therapy of metastatic renal cell carcinoma: the role of interleukin-2. Cancer, 80: 1198-1220, 1997.
20. Robson, C. J., Churchill, B. M., and Anderson, W. The results of radical nephrectomy for renal cell carcinoma. J.Urol., 101: 297-301, 1969.
21. Guinan, P., Sobin, L. H., Algaba, F., Badellino, F., Kameyama, S., MacLennan, G., and Novick, A. TNM staging of renal cell carcinoma: Workgroup No. 3. Union International Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer, 80: 992-993, 1997.
22. Javidan, J., Stricker, H. J., Tamboli, P., Amin, M. B., Peabody, J. O., Deshpande, A., Menon, M., and Amin, M. B. Prognostic significance of the 1997 TNM classification of renal cell carcinoma. J.Urol., 162: 1277-1281, 1999.
23. Haas, G. P., Hillman, G. G., Redman, B. G., and Pontes, J. E. Immunotherapy of renal cell carcinoma. CA Cancer J.Clin., 43: 177-187, 1993.
24. Patel, N. P. and Lavengood, R. W. Renal cell carcinoma: natural history and results of treatment. J.Urol., 119 : 722-726, 1978.
25. Elson, P. J., Witte, R. S., and Trump, D. L. Prognostic factors for survival in patients with recurrent or metastatic renal cell carcinoma. Cancer Res., 48: 7310-7313, 1988.
26. Hoffman, D. M. and Figlin, R. A. Natural history and prognostic factors associated with metastatic renal cell carcinoma. In Renal & adrenal tumors, edited by Arie Belldegrun, 351-358. Oxford university press 2003.
27. Cooney, M. M., Remick, S. C., and Vogelzang, N. J. A medical oncologist's approach to immunotherapy for advanced renal tumors: is nephrectomy indicated? Curr.Urol.Rep., 5: 19-24, 2004.
28. Motzer, R. J., Bander, N. H., and Nanus, D. M. Renal-cell carcinoma. N.Engl.J Med., 335: 865-875, 1996.
29. Gettman, M. T. and Blute, M. L. Surgical management of renal cell carcinoma invading the vena cava. Curr.Urol.Rep., 3: 37-43, 2002.
30. Alamdari, F. I. and Ljungberg, B. Adrenal metastasis in renal cell carcinoma: A recommendation for adjustment of the TNM staging system. Scand.J.Urol.Nephrol., 39: 277-282, 2005.
31. Canfield, S. E., Kamat, A. M., Sanchez-Ortiz, R. F., Detry, M., Swanson, D. A., and Wood, C. G. Renal cell carcinoma with nodal metastases in the absence of distant metastatic disease (clinical stage TxN1-2M0): the impact of aggressive surgical resection on patient outcome. J Urol., 175: 864-869, 2006.
32. Blom, J. H., van Poppel, H., Marechal, J. M., Jacqmin, D., Sylvester, R., Schroder, F. H., and de Prijck, L. Radical nephrectomy with and without lymph node dissection: preliminary results of the EORTC randomized phase III protocol 30881. EORTC Genitourinary Group. Eur.Urol., 36: 570-575, 1999.
33. Pantuck, A. J., Zisman, A., Dorey, F., Chao, D. H., Han, K. R., Said, J., Gitlitz, B. J., Figlin, R. A., and Belldegrun, A. S. Renal cell carcinoma with retroperitoneal lymph nodes: role of lymph node dissection. J.Urol., 169: 2076-2083, 2003.
34. Larsen, E. H., Frimodt-Møller, P. C., Horn, T., Dorph, S., and von der Maase, H. Nyrecancer. Klaringsrapport nr. 7, 2002. Betænkning fra arbejdsgruppe nedsat af Dansk Urologisk Selskab. www.ugeskriftet.dk, 2002.
35. Novick, A. C. Laparoscopic and partial nephrectomy. Clin.Cancer Res., 10: 6322S-6327S, 2004.
36. Hines-Peralta, A. and Goldberg, S. N. Review of radiofrequency ablation for renal cell carcinoma. Clin.Cancer Res., 10: 6328S-6334S, 2004.
37. Werf-Messing, B. Proceedings: Carcinoma of the kidney. Cancer, 32: 1056-1061, 1973.
38. Juusela, H., Malmio, K., Alfthan, O., and Oravisto, K. J. Preoperative irradiation in the treatment of renal adenocarcinoma. Scand.J.Urol.Nephrol., 11: 277-281, 1977.
39. Finney, R. The value of radiotherapy in the treatment of hypernephroma – a clinical trial. Br.J.Urol., 45: 258-269, 1973.
40. Kjaer, M., Frederiksen, P. L., and Engelholm, S. A. Postoperative radiotherapy in stage II and III renal adenocarcinoma. A randomized trial by the Copenhagen Renal Cancer Study Group. Int.J.Radiat.Oncol.Biol.Phys., 13: 665-672, 1987.
41. Jocham, D., Richter, A., Hoffmann, L., Iwig, K., Fahlenkamp, D., Zakrzewski, G., Schmitt, E., Dannenberg, T., Lehmacher, W., von Wietersheim, J., and Doehn, C. Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. Lancet, 363: 594-599, 2004.
42. Pizzocaro, G., Piva, L., Di Fronzo, G., Giongo, A., Cozzoli, A., Dormia, E., Minervini, S., Zanollo, A., Fontanella, U., Longo, G., and . Adjuvant medroxyprogesterone acetate to radical nephrectomy in renal cancer: 5-year results of a prospective randomized study. J.Urol., 138: 1379-1381, 1987.
43. Pizzocaro, G., Piva, L., Colavita, M., Ferri, S., Artusi, R., Boracchi, P., Parmiani, G., and Marubini, E. Interferon adjuvant to radical nephrectomy in Robson stages II and III renal cell carcinoma: a multicentric randomized study. J.Clin.Oncol., 19: 425-431, 2001.
44. Messing, E. M., Manola, J., Wilding, G., Propert, K., Fleischmann, J., Crawford, E. D., Pontes, J. E., Hahn, R., and Trump, D. Phase III study of interferon alfa-NL as adjuvant treatment for resectable renal cell carcinoma: an Eastern Cooperative Oncology Group/Intergroup trial. J.Clin.Oncol., 21: 1214-1222, 2003.
45. Clark, J. I., Atkins, M. B., Urba, W. J., Creech, S., Figlin, R. A., Dutcher, J. P., Flaherty, L., Sosman, J. A., Logan, T. F., White, R., Weiss, G. R.,

Redman, B. G., Tretter, C. P., McDermott, D., Smith, J. W., Gordon, M. S., and Margolin, K. A. Adjuvant high-dose bolus interleukin-2 for patients with high-risk renal cell carcinoma: a cytokine working group randomized trial. J.Clin.Oncol., 21: 3133-3140, 2003.
46. Atzpodien, J., Schmitt, E., Gertenbach, U., Fornara, P., Heynemann, H., Maskow, A., Ecke, M., Woltjen, H. H., Jentsch, H., Wieland, W., Wandert, T., and Reitz, M. Adjuvant treatment with interleukin-2- and interferon-alpha2a-based chemoimmunotherapy in renal cell carcinoma post tumour nephrectomy: results of a prospectively randomised trial of the German Cooperative Renal Carcinoma Chemoimmunotherapy Group (DGCIN). Br.J.Cancer, 92: 843-846, 2005.
47. Barney, J. and Churchill, E. Adenocarcinoma of the kidney with metastasis to the lung: Cured by nephrectomy and lobectomy. J Urol., 42: 269-276, 1939.
48. Kavolius, J. P., Mastorakos, D. P., Pavlovich, C., Russo, P., Burt, M. E., and Brady, M. S. Resection of metastatic renal cell carcinoma. J.Clin. Oncol., 16: 2261-2266, 1998.
49. Freed, S. Z., Halperin, J. P., and Gordon, M. Idiopathic regression of metastases from renal cell carcinoma. J.Urol., 118: 538-542, 1977.
50. Gleave, M. E., Elhilali, M., Fradet, Y., Davis, I., Venner, P., Saad, F., Klotz, L. H., Moore, M. J., Paton, V., and Bajamonde, A. Interferon gamma-1b compared with placebo in metastatic renal-cell carcinoma. Canadian Urologic Oncology Group. N.Engl.J.Med., 338: 1265-1271, 1998.
51. Belldegrun, A., Shvarts, O., and Figlin, R. A. Expanding the indications for surgery and adjuvant interleukin-2-based immunotherapy in patients with advanced renal cell carcinoma. Cancer J.Sci.Am., 6 Suppl 1: S88-S92, 2000.
52. Rackley, R., Novick, A., Klein, E., Bukowski, R., McLain, D., and Goldfarb, D. The impact of adjuvant nephrectomy on multimodality treatment of metastatic renal cell carcinoma. J.Urol., 152: 1399-1403, 1994.
53. Bennett, R. T., Lerner, S. E., Taub, H. C., Dutcher, J. P., and Fleischmann, J. Cytoreductive surgery for stage IV renal cell carcinoma. J.Urol., 154: 32-34, 1995.
54. Walther, M. M., Yang, J. C., Pass, H. I., Linehan, W. M., and Rosenberg, S. A. Cytoreductive surgery before high dose interleukin-2 based therapy in patients with metastatic renal cell carcinoma. J.Urol., 158: 1675-1678, 1997.
55. Mickisch, G. H., Garin, A., van Poppel, H., de Prijck, L., and Sylvester, R. Radical nephrectomy plus interferon-alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. Lancet, 358: 966-970, 2001.
56. Flanigan, R. C., Salmon, S. E., Blumenstein, B. A., Bearman, S. I., Roy, V., McGrath, P. C., Caton, J. R., Jr., Munshi, N., and Crawford, E. D. Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. N.Engl.J.Med., 345: 1655-1659, 2001.
57. Flanigan, R. C., Mickisch, G., Sylvester, R., Tangen, C., van Poppel, H., and Crawford, E. D. Cytoreductive nephrectomy in patients with metastatic renal cancer: a combined analysis. J.Urol., 171: 1071-1076, 2004.
58. Flanigan, R. C. Debulking nephrectomy in metastatic renal cancer. Clin.Cancer Res., 10: 6335S-6341S, 2004.
59. Tannock, I. F. Commentary on "cytoreduction nephrectomy in metastatic renal cancer: the results of Southwest Oncology Group Trial 8949". J Clin.Oncol., 18: 39S-42S, 2000.
60. Motzer, R. J. and Russo, P. Systemic therapy for renal cell carcinoma. J.Urol., 163: 408-417, 2000.
61. Yagoda, A., Abi-Rached, B., and Petrylak, D. Chemotherapy for advanced renal-cell carcinoma: 1983-1993. Semin.Oncol., 22: 42-60, 1995.
62. Chapman, A. E. and Goldstein, L. J. Multiple drug resistance: biologic basis and clinical significance in renal-cell carcinoma. Semin.Oncol., 22: 17-28, 1995.
63. Amato, R. J. Chemotherapy for renal cell carcinoma. Semin.Oncol., 27: 177-186, 2000.
64. Elhilali, M. M., Gleave, M., Fradet, Y., Davis, I., Venner, P., Saad, F., Klotz, L., Moore, R., Ernst, S., and Paton, V. Placebo-associated remissions in a multicentre, randomized, double-blind trial of interferon gamma-1b for the treatment of metastatic renal cell carcinoma. The Canadian Urologic Oncology Group. BJU.Int., 86: 613-618, 2000.
65. Oliver, R. T., Miller, R. M., Mehta, A., and Barnett, M. J. A phase 2 study of surveillance in patients with metastatic renal cell carcinoma and assessment of response of such patients to therapy on progression. Mol.Biother., 1: 14-20, 1988.
66. Snow, R. M. and Schellhammer, P. F. Spontaneous regression of metastatic renal cell carcinoma. Urology, 20: 177-181, 1982.
67. Vogelzang, N. J., Priest, E. R., and Borden, L. Spontaneous regression of histologically proved pulmonary metastases from renal cell carcinoma: a case with 5-year followup. J Urol., 148: 1247-1248, 1992.
68. Finke, J. H., Tubbs, R., Connelly, B., Pontes, E., and Montie, J. Tumor-infiltrating lymphocytes in patients with renal-cell carcinoma. Ann.N.Y.Acad.Sci., 532: 387-394, 1988.
69. Vogelzang, N. J. and Stadler, W. M. Kidney cancer. Lancet, 352: 1691-1696, 1998.
70. Pfeffer, L. M., Dinarello, C. A., Herberman, R. B., Williams, B. R., Borden, E. C., Bordens, R., Walter, M. R., Nagabhushan, T. L., Trotta, P. P., and Pestka, S. Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res., 58: 2489-2499, 1998.
71. Quesada, J. R., Swanson, D. A., Trindade, A., and Gutterman, J. U. Renal cell carcinoma: antitumor effects of leukocyte interferon. Cancer Res., 43: 940-947, 1983.
72. Nanus, D. M., Pfeffer, L. M., Bander, N. H., Bahri, S., and Albino, A. P. Antiproliferative and antitumor effects of alpha-interferon in renal cell carcinomas: correlation with the expression of a kidney-associated differentiation glycoprotein. Cancer Res., 50: 4190-4194, 1990.
73. Steineck, G., Strander, H., Carbin, B. E., Borgstrom, E., Wallin, L., Achtnich, U., Arvidsson, A., Soderlund, V., Naslund, I., Esposti, P. L., and . Recombinant leukocyte interferon alpha-2a and medroxyprogesterone in advanced renal cell carcinoma. A randomized trial. Acta Oncol., 29: 155-162, 1990.
74. Kriegmair, M., Oberneder, R., and Hofstetter, A. Interferon alfa and vinblastine versus medroxyprogesterone acetate in the treatment of metastatic renal cell carcinoma. Urology, 45: 758-762, 1995.
75. Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Medical Research Council Renal Cancer Collaborators. Lancet, 353: 14-17, 1999.
76. Pyrhonen, S., Salminen, E., Ruutu, M., Lehtonen, T., Nurmi, M., Tammela, T., Juusela, H., Rintala, E., Hietanen, P., and Kellokumpu-Lehtinen, P. L. Prospective randomized trial of interferon alfa-2a plus vinblastine versus vinblastine alone in patients with advanced renal cell cancer. J.Clin.Oncol., 17: 2859-2867, 1999.
77. Coppin, C., Porzsolt, F., Awa, A., Kumpf, J., Coldman, A., and Wilt, T. Immunotherapy for advanced renal cell cancer. Cochrane.Database.Syst.Rev., CD001425, 2005.
78. Minasian, L. M., Motzer, R. J., Gluck, L., Mazumdar, M., Vlamis, V., and Krown, S. E. Interferon alfa-2a in advanced renal cell carcinoma: treatment results and survival in 159 patients with long-term follow-up. J.Clin.Oncol., 11: 1368-1375, 1993.
79. Morgan, D. A., Ruscetti, F. W., and Gallo, R. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science, 193: 1007-1008, 1976.
80. Bazan, J. F. Unraveling the structure of IL-2. Science, 257: 410-413, 1992.
81. Rosenberg, S. A. Progress in human tumour immunology and immunotherapy. Nature, 411: 380-384, 2001.
82. Schmidinger, M., Hejna, M., and Zielinski, C. C. Aldesleukin in advanced renal cell carcinoma. Expert.Rev.Anticancer Ther., 4: 957-980, 2004.
83. Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., and . Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N.Engl.J.Med., 313: 1485-1492, 1985.
84. Fyfe, G., Fisher, R. I., Rosenberg, S. A., Sznol, M., Parkinson, D. R., and Louie, A. C. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J.Clin.Oncol., 13: 688-696, 1995.
85. Fisher, R. I., Rosenberg, S. A., and Fyfe, G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. Cancer J.Sci.Am., 6 Suppl 1: S55-S57, 2000.
86. von der Maase, H., Geertsen, P., Thatcher, N., Jasmin, C., Mercatello, A., Fossa, S. D., Symann, M., Stoter, G., Nagel, G., Israel, L., and . Recombinant interleukin-2 in metastatic renal cell carcinoma – a European multicentre phase II study. Eur.J.Cancer, 27: 1583-1589, 1991.
87. Atzpodien, J., Korfer, A., Franks, C. R., Poliwoda, H., and Kirchner, H. Home therapy with recombinant interleukin-2 and interferon-alpha 2b in advanced human malignancies. Lancet, 335: 1509-1512, 1990.
88. Negrier, S., Escudier, B., Lasset, C., Douillard, J. Y., Savary, J., Chevreau, C., Ravaud, A., Mercatello, A., Peny, J., Mousseau, M., Philip, T., and Tursz, T. Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d'Immunotherapie. N.Engl.J.Med., 338: 1272-1278, 1998.
89. Yang, J. C., Sherry, R. M., Steinberg, S. M., Topalian, S. L., Schwartzentruber, D. J., Hwu, P., Seipp, C. A., Rogers-Freezer, L., Morton, K. E., White, D. E., Liewehr, D. J., Merino, M. J., and Rosenberg, S. A. Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer. J.Clin.Oncol., 21: 3127-3132, 2003.
90. McDermott, D. F., Regan, M. M., Clark, J. I., Flaherty, L. E., Weiss, G. R., Logan, T. F., Kirkwood, J. M., Gordon, M. S., Sosman, J. A., Ernstoff, M. S., Tretter, C. P., Urba, W. J., Smith, J. W., Margolin, K. A., Mier, J. W., Gollob, J. A., Dutcher, J. P., and Atkins, M. B. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J.Clin. Oncol., 23: 133-141, 2005.
91. Negrier, S., Maral, J., Drevon, M., Vinke, J., Escudier, B., and Philip, T.

Long-term follow-up of patients with metastatic renal cell carcinoma treated with intravenous recombinant interleukin-2 in Europe. Cancer J.Sci.Am., 6 Suppl 1: S93-S98, 2000.
92. Negrier, S., Perol, D and others. Do cytokines improve survival in patients with metastatic renal cell carcinoma (MRCC) of intermediate prognosis? Results of the prospective randomized PERCY Quattro trial. (ASCO 2005, Late Breaking Abstract 4511).
93. Henriksson, R., Nilsson, S., Colleen, S., Wersall, P., Helsing, M., Zimmerman, R., and Engman, K. Survival in renal cell carcinoma-a randomized evaluation of tamoxifen vs interleukin 2, alpha-interferon (leucocyte) and tamoxifen. Br.J Cancer, 77: 1311-1317, 1998.
94. Bordin, V., Giani, L., Meregalli, S., Bukovec, R., Vaghi, M. M., Mandala, M., Paolorossi, F., Ardizzoia, A., Tancini, G., Barni, S., Frigerio, F., Fumagalli, L., Bordoni, A., Valsuani, G., Di Felice, G., and Lissoni, P. Five-year survival results of subcutaneous low-dose immunotherapy with interleukin-2 alone in metastatic renal cell cancer patients. Urol.Int., 64: 3-8, 2000.
95. Atzpodien, J., Hoffmann, R., Franzke, M., Stief, C., Wandert, T., and Reitz, M. Thirteen-year, long-term efficacy of interferon 2alpha and interleukin 2-based home therapy in patients with advanced renal cell carcinoma. Cancer, 95: 1045-1050, 2002.
96. Geertsen, P. F., Hermann, G. G., von der Maase, H., and Steven, K. Treatment of metastatic renal cell carcinoma by continuous intravenous infusion of recombinant interleukin-2: a single-center phase II study. J.Clin.Oncol., 10: 753-759, 1992.
97. Law, T. M., Motzer, R. J., Mazumdar, M., Sell, K. W., Walther, P. J., O'Connell, M., Khan, A., Vlamis, V., Vogelzang, N. J., and Bajorin, D. F. Phase III randomized trial of interleukin-2 with or without lymphokine-activated killer cells in the treatment of patients with advanced renal cell carcinoma. Cancer, 76: 824-832, 1995.
98. Figlin, R. A., Thompson, J. A., Bukowski, R. M., Vogelzang, N. J., Novick, A. C., Lange, P., Steinberg, G. D., and Belldegrun, A. S. Multicenter, randomized, phase III trial of CD8(+) tumor-infiltrating lymphocytes in combination with recombinant interleukin-2 in metastatic renal cell carcinoma. J.Clin.Oncol., 17: 2521-2529, 1999.
99. Avigan, D. Dendritic cell-tumor fusion vaccines for renal cell carcinoma. Clin.Cancer Res., 10: 6347S-6352S, 2004.
100. Bleumer, I., Knuth, A., Oosterwijk, E., Hofmann, R., Varga, Z., Lamers, C., Kruit, W., Melchior, S., Mala, C., Ullrich, S., De Mulder, P., Mulders, P. F., and Beck, J. A phase II trial of chimeric monoclonal antibody G250 for advanced renal cell carcinoma patients. Br.J.Cancer, 90: 985-990, 2004.
101. Escudier, B., Lassau, N., Couanet, D., Angevin, E., Mesrati, F., Leborgne, S., Garofano, A., Leboulaire, C., Dupouy, N., and Laplanche, A. Phase II trial of thalidomide in renal-cell carcinoma. Ann.Oncol., 13: 1029-1035, 2002.
102. Motzer, R. J., Berg, W., Ginsberg, M., Russo, P., Vuky, J., Yu, R., Bacik, J., and Mazumdar, M. Phase II trial of thalidomide for patients with advanced renal cell carcinoma. J.Clin.Oncol., 20: 302-306, 2002.
103. Motzer, R. J., Murphy, B. A., Bacik, J., Schwartz, L. H., Nanus, D. M., Mariani, T., Loehrer, P., Wilding, G., Fairclough, D. L., Cella, D., and Mazumdar, M. Phase III trial of interferon alfa-2a with or without 13-cis-retinoic acid for patients with advanced renal cell carcinoma. J.Clin.Oncol., 18: 2972-2980, 2000.
104. Aass, N., de Mulder, P. H., Mickisch, G. H., Mulders, P., van Oosterom, A. T., van Poppel, H., Fossa, S. D., de Prijck, L., and Sylvester, R. J. Randomized phase II/III trial of interferon Alfa-2a with and without 13-cis-retinoic acid in patients with progressive metastatic renal cell Carcinoma: the European Organisation for Research and Treatment of Cancer Genito-Urinary Tract Cancer Group (EORTC 30951). J.Clin.Oncol., 23: 4172-4178, 2005.
105. Childs, R., Chernoff, A., Contentin, N., Bahceci, E., Schrump, D., Leitman, S., Read, E. J., Tisdale, J., Dunbar, C., Linehan, W. M., Young, N. S., and Barrett, A. J. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N.Engl.J.Med., 343: 750-758, 2000.
106. Takahashi, Y. and Childs, R. W. Nonmyeloablative transplantation: an allogeneic-based immunotherapy for renal cell carcinoma. Clin.Cancer Res., 10: 6353S-6359S, 2004.
107. van Spronsen, D. J., Mulders, P. F., and de Mulder, P. H. Novel treatments for metastatic renal cell carcinoma. Crit Rev.Oncol.Hematol., 55: 177-191, 2005.
108. Yang, J. C. Bevacizumab for patients with metastatic renal cancer: an update. Clin.Cancer Res., 10: 6367S-6370S, 2004.
109. Potti, A. and George, D. J. Tyrosine kinase inhibitors in renal cell carcinoma. Clin.Cancer Res., 10: 6371S-6376S, 2004.
110. Gordon, M. S. Novel antiangiogenic therapies for renal cell cancer. Clin.Cancer Res., 10: 6377S-6381S, 2004.
111. Dutcher, J. P. Mammalian target of rapamycin inhibition. Clin.Cancer Res., 10: 6382S-6387S, 2004.
112. Ahmad, T. and Eisen, T. Kinase inhibition with BAY 43-9006 in renal cell carcinoma. Clin.Cancer Res., 10: 6388S-6392S, 2004.
113. Yang, J. C., Haworth, L., Sherry, R. M., Hwu, P., Schwartzentruber, D. J., Topalian, S. L., Steinberg, S. M., Chen, H. X., and Rosenberg, S. A. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N.Engl.J.Med., 349: 427-434, 2003.
114. Hainsworth, J. D., Sosman, J. A., Spigel, D. R., Edwards, D. L., Baughman, C., and Greco, A. Treatment of metastatic renal cell carcinoma with a combination of bevacizumab and erlotinib. J Clin.Oncol., 23: 7889-7896, 2005.
115. Motzer, R. J., Michaelson, M. D., Redman, B. G., Hudes, G. R., Wilding, G., Figlin, R. A., Ginsberg, M. S., Kim, S. T., Baum, C. M., DePrimo, S. E., Li, J. Z., Bello, C. L., Theuer, C. P., George, D. J., and Rini, B. I. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin.Oncol., 24: 16-24, 2006.
116. Smith, K. A. Interleukin-2: inception, impact, and implications. Science, 240: 1169-1176, 1988.
117. Hokland, M. and Kuppen, P. J. Natural killer cells: from "disturbing" background to central players of immune responses. Mol.Immunol., 42: 381-383, 2005.
118. Farag, S. S. and Caligiuri, M. A. Human natural killer cell development and biology. Blood Rev., 20:123-37, 2006.
119. Mingari, M. C., Gerosa, F., Carra, G., Accolla, R. S., Moretta, A., Zubler, R. H., Waldmann, T. A., and Moretta, L. Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. Nature, 312: 641-643, 1984.
120. Espinoza-Delgado, I., Bosco, M. C., Musso, T., Gusella, G. L., Longo, D. L., and Varesio, L. Interleukin-2 and human monocyte activation. J Leukoc.Biol., 57: 13-19, 1995.
121. Ferrante, A. Activation of neutrophils by interleukins-1 and -2 and tumor necrosis factors. Immunol.Ser., 57: 417-436, 1992.
122. Fehniger, T. A., Cooper, M. A., and Caligiuri, M. A. Interleukin-2 and interleukin-15: immunotherapy for cancer. Cytokine Growth Factor Rev., 13: 169-183, 2002.
123. Jeal, W. and Goa, K. L. Aldesleukin (Recombinant Interleukin-2). A review of its Pharmacological Properties, Clinical Efficacy and Tolerability in Patients with Renal Cell Carcinoma. BioDrugs, 7: 285-317, 1997.
124. Carlos, T. M. Leukocyte recruitment at sites of tumor: dissonant orchestration. J Leukoc.Biol., 70: 171-184, 2001.
125. Sogn, J. A. Tumor immunology: the glass is half full. Immunity, 9: 757-763, 1998.
126. Pardoll, D. Does the immune system see tumors as foreign or self? Annu.Rev.Immunol., 21: 807-839, 2003.
127. van der, B. P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den, E. B., Knuth, A., and Boon, T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science, 254: 1643-1647, 1991.
128. Rosenberg, S. A. Shedding light on immunotherapy for cancer. N.Engl.J Med., 350: 1461-1463, 2004.
129. Michael, A. and Pandha, H. S. Renal-cell carcinoma: tumour markers, T-cell epitopes, and potential for new therapies. Lancet Oncol., 4: 215-223, 2003.
130. Novellino, L., Castelli, C., and Parmiani, G. A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol.Immunother., 54: 187-207, 2005.
131. Schmidt, S. M., Schag, K., Muller, M. R., Weinschenk, T., Appel, S., Schoor, O., Weck, M. M., Grunebach, F., Kanz, L., Stevanovic, S., Rammensee, H. G., and Brossart, P. Induction of adipophilin-specific cytotoxic T lymphocytes using a novel HLA-A2-binding peptide that mediates tumor cell lysis. Cancer Res., 64: 1164-1170, 2004.
132. Hanada, K., Yewdell, J. W., and Yang, J. C. Immune recognition of a human renal cancer antigen through post-translational protein splicing. Nature, 427: 252-256, 2004.
133. Vissers, J. L., De Vries, I. J., Schreurs, M. W., Engelen, L. P., Oosterwijk, E., Figdor, C. G., and Adema, G. J. The renal cell carcinoma-associated antigen G250 encodes a human leukocyte antigen (HLA)-A2.1-restricted epitope recognized by cytotoxic T lymphocytes. Cancer Res., 59: 5554-5559, 1999.
134. Ronsin, C., Chung-Scott, V., Poullion, I., Aknouche, N., Gaudin, C., and Triebel, F. A non-AUG-defined alternative open reading frame of the intestinal carboxyl esterase mRNA generates an epitope recognized by renal cell carcinoma-reactive tumor-infiltrating lymphocytes in situ. J Immunol., 163: 483-490, 1999.
135. Probst-Kepper, M., Stroobant, V., Kridel, R., Gaugler, B., Landry, C., Brasseur, F., Cosyns, J. P., Weynand, B., Boon, T., and Van Den Eynde, B. J. An alternative open reading frame of the human macrophage colony-stimulating factor gene is independently translated and codes for an antigenic peptide of 14 amino acids recognized by tumor-infiltrating CD8 T lymphocytes. J Exp.Med., 193: 1189-1198, 2001.
136. Neumann, E., Engelsberg, A., Decker, J., Storkel, S., Jaeger, E., Huber, C., and Seliger, B. Heterogeneous expression of the tumor-associated antigens RAGE-1, PRAME, and glycoprotein 75 in human renal cell

carcinoma: candidates for T-cell-based immunotherapies? Cancer Res., 58: 4090-4095, 1998.
137. Bui, M. H., Seligson, D., Han, K. R., Pantuck, A. J., Dorey, F. J., Huang, Y., Horvath, S., Leibovich, B. C., Chopra, S., Liao, S. Y., Stanbridge, E., Lerman, M. I., Palotie, A., Figlin, R. A., and Belldegrun, A. S. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin.Cancer Res., 9: 802-811, 2003.
138. Atkins, M., Regan, M., McDermott, D., Mier, J., Stanbridge, E., Youmans, A., Febbo, P., Upton, M., Lechpammer, M., and Signoretti, S. Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer. Clin.Cancer Res., 11: 3714-3721, 2005.
139. Foss, F. M. Immunologic mechanisms of antitumor activity. Semin.Oncol., 29: 5-11, 2002.
140. Britten, C. M., Gouttefangeas, C., and Kreiter, S. Cancer immunotherapy 2005: Mainz, Germany, 12-13 May 2005. Cancer Immunol.Immunother., 55: 475-480, 2006.
141. Malmberg, K. J. Effective immunotherapy against cancer: a question of overcoming immune suppression and immune escape? Cancer Immunol.Immunother., 53: 879-892, 2004.
142. Malmberg, K. J. and Ljunggren, H. G. Escape from immune- and non-immune-mediated tumor surveillance. Semin.Cancer Biol., 2005.
143. Takahashi, A., Hanson, M. G., Norell, H. R., Havelka, A. M., Kono, K., Malmberg, K. J., and Kiessling, R. V. Preferential cell death of CD8+ effector memory (CCR7-CD45RA-) T cells by hydrogen peroxide-induced oxidative stress. J.Immunol., 174: 6080-6087, 2005.
144. O'Byrne, K. J. and Dalgleish, A. G. Chronic immune activation and inflammation as the cause of malignancy. Br.J.Cancer, 85: 473-483, 2001.
145. Coussens, L. M. and Werb, Z. Inflammation and cancer. Nature, 420: 860-867, 2002.
146. Seaman, W. E., Gindhart, T. D., Blackman, M. A., Dalal, B., Talal, N., and Werb, Z. Suppression of natural killing in vitro by monocytes and polymorphonuclear leukocytes: requirement for reactive metabolites of oxygen. J.Clin.Invest, 69: 876-888, 1982.
147. Hansson, M., Asea, A., Ersson, U., Hermodsson, S., and Hellstrand, K. Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites. J.Immunol., 156: 42-47, 1996.
148. Hellstrand, K., Asea, A., Dahlgren, C., and Hermodsson, S. Histaminergic regulation of NK cells. Role of monocyte-derived reactive oxygen metabolites. J.Immunol., 153: 4940-4947, 1994.
149. Malmberg, K. J., Arulampalam, V., Ichihara, F., Petersson, M., Seki, K., Andersson, T., Lenkei, R., Masucci, G., Pettersson, S., and Kiessling, R. Inhibition of activated/memory (CD45RO(+)) T cells by oxidative stress associated with block of NF-kappaB activation. J.Immunol., 167: 2595-2601, 2001.
150. Kono, K., Salazar-Onfray, F., Petersson, M., Hansson, J., Masucci, G., Wasserman, K., Nakazawa, T., Anderson, P., and Kiessling, R. Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell- and natural killer cell-mediated cytotoxicity. Eur.J.Immunol., 26: 1308-1313, 1996.
151. Tartour, E., Latour, S., Mathiot, C., Thiounn, N., Mosseri, V., Joyeux, I., D'Enghien, C. D., Lee, R., Debre, B., and Fridman, W. H. Variable expression of CD3-zeta chain in tumor-infiltrating lymphocytes (TIL) derived from renal-cell carcinoma: relationship with TIL phenotype and function. Int.J.Cancer, 63: 205-212, 1995.
152. Finke, J. H., Zea, A. H., Stanley, J., Longo, D. L., Mizoguchi, H., Tubbs, R. R., Wiltrout, R. H., O'Shea, J. J., Kudoh, S., and Klein, E. Loss of T-cell receptor zeta chain and p56lck in T-cells infiltrating human renal cell carcinoma. Cancer Res., 53: 5613-5616, 1993.
153. Hellstrand, K. and Hermodsson, S. Synergistic activation of human natural killer cell cytotoxicity by histamine and interleukin-2. Int.Arch.Allergy Appl.Immunol., 92: 379-389, 1990.
154. Hellstrand, K. Histamine in cancer immunotherapy: a preclinical background. Semin.Oncol, 29: 35-40, 2002.
155. Hellstrand, K. and Hermodsson, S. Histamine H2-receptor-mediated regulation of human natural killer cell activity. J.Immunol., 137: 656-660, 1986.
156. Betten, A., Dahlgren, C., Hermodsson, S., and Hellstrand, K. Histamine inhibits neutrophil NADPH oxidase activity triggered by the lipoxin A4 receptor-specific peptide agonist Trp-Lys-Tyr-Met-Val-Met. Scand.J.Immunol., 58: 321-326, 2003.
157. Hellstrand, K., Asea, A., and Hermodsson, S. Role of histamine in natural killer cell-mediated resistance against tumor cells. J.Immunol., 145: 4365-4370, 1990.
158. Asea, A., Hermodsson, S., and Hellstrand, K. Histaminergic regulation of natural killer cell-mediated clearance of tumour cells in mice. Scand.J.Immunol., 43: 9-15, 1996.
159. Miller, A. B., Hoogstraten, B., Staquet, M., and Winkler, A. Reporting results of cancer treatment. Cancer, 47: 207-214, 1981.
160. Jennings, P. E., Donald, J. J., Coral, A., Rode, J., and Lees, W. R. Ultrasound-guided core biopsy. Lancet, 1 : 1369-1371, 1989.
161. Shi, S. R., Key, M. E., and Kalra, K. L. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem.Cytochem., 39: 741-748, 1991.
162. Miller, R. T., Swanson, P. E., and Wick, M. R. Fixation and epitope retrieval in diagnostic immunohistochemistry: a concise review with practical considerations. Appl.Immunohistochem.Mol.Morphol., 8: 228-235, 2000.
163. Sabattini, E., Bisgaard, K., Ascani, S., Poggi, S., Piccioli, M., Ceccarelli, C., Pieri, F., Fraternali-Orcioni, G., and Pileri, S. A. The EnVision++ system: a new immunohistochemical method for diagnostics and research. Critical comparison with the APAAP, ChemMate, CSA, LABC, and SABC techniques. J Clin.Pathol., 51: 506-511, 1998.
164. Gundersen, H. J., Bendtsen, T. F., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sorensen, F. B., and Vesterby, A. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS, 96: 379-394, 1988.
165. Tandrup, T. Unbiased estimates of number and size of rat dorsal root ganglion cells in studies of structure and cell survival. J Neurocytol., 33: 173-192, 2004.
166. Gundersen, H. J. and Jensen, E. B. The efficiency of systematic sampling in stereology and its prediction. J Microsc., 147 (Pt 3): 229-263, 1987.
167. Gundersen, H. J. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc., 143 (Pt 1): 3-45, 1986.
168. Petersen, M. S., Petersen, C. C., Agger, R., Hokland, M., and Gundersen, H. J. A simple method for unbiased quantitation of adoptively transferred cells in solid tissues. J Immunol.Methods, 309:173-81, 2006.
169. Hakansson, A., Gustafsson, B., Krysander, L., and Hakansson, L. Effect of IFN-alpha on tumor-infiltrating mononuclear cells and regressive changes in metastatic malignant melanoma. J Interferon Cytokine Res., 18: 33-39, 1998.
170. Hansen, S., Grabau, D. A., Rose, C., Bak, M., and Sorensen, F. B. Angiogenesis in breast cancer: a comparative study of the observer variability of methods for determining microvessel density. Lab Invest, 78: 1563-1573, 1998.
171. Jensen, V., Sorensen, F. B., Bentzen, S. M., Ladekarl, M., Nielsen, O. S., Keller, J., and Jensen, O. M. Proliferative activity (MIB-1 index) is an independent prognostic parameter in patients with high-grade soft tissue sarcomas of subtypes other than malignant fibrous histiocytomas: a retrospective immunohistological study including 216 soft tissue sarcomas. Histopathology, 32: 536-546, 1998.
172. Wallace, D., Hildesheim, A., and Pinto, L. A. Comparison of benchtop microplate beta counters with the traditional gamma counting method for measurement of chromium-51 release in cytotoxic assays. Clin.Diagn.Lab Immunol., 11: 255-260, 2004.
173. Hokland, P, Bonde, J and others. Isolation of Mononuclear cells from Human Blood and Bone Marrow and Identifications of Leukocytes Subsets by Multiparameter flow Cytometry. Cell Biology (Edited by Celis, JE), 164-169.
174. Rosenberg, S. A., Yang, J. C., and Restifo, N. P. Cancer immunotherapy: moving beyond current vaccines. Nat.Med., 10: 909-915, 2004.
175. Bukowski, R. M., Olencki, T., Wang, Q., Peereboom, D., Budd, G. T., Elson, P., Sandstrom, K., Tuason, L., Rayman, P., Tubbs, R., McLain, D., Klein, E., Novick, A., and Finke, J. Phase II trial of interleukin-2 and interferon-alpha in patients with renal cell carcinoma: clinical results and immunologic correlates of response. J.Immunother., 20: 301-311, 1997.
176. Cohen, P. J., Lotze, M. T., Roberts, J. R., Rosenberg, S. A., and Jaffe, E. S. The immunopathology of sequential tumor biopsies in patients treated with interleukin-2. Correlation of response with T-cell infiltration and HLA-DR expression. Am.J.Pathol., 129: 208-216, 1987.
177. Rubin, J. T., Elwood, L. J., Rosenberg, S. A., and Lotze, M. T. Immunohistochemical correlates of response to recombinant interleukin-2-based immunotherapy in humans. Cancer Res., 49: 7086-7092, 1989.
178. Hernberg, M. Lymphocyte subsets as prognostic markers for cancer patients receiving immunomodulative therapy. Med.Oncol., 16: 145-153, 1999.
179. Fumagalli, L. A., Vinke, J., Hoff, W., Ypma, E., Brivio, F., and Nespoli, A. Lymphocyte counts independently predict overall survival in advanced cancer patients: a biomarker for IL-2 immunotherapy. J Immunother., 26: 394-402, 2003.
180. Hakansson, A., Gustafsson, B., Krysander, L., and Hakansson, L. Tumour-infiltrating lymphocytes in metastatic malignant melanoma and response to interferon alpha treatment. Br.J.Cancer, 74: 670-676, 1996.
181. Hakansson, A., Gustafsson, B., Krysander, L., Hjelmqvist, B., Rettrup, B., and Hakansson, L. Biochemotherapy of metastatic malignant melanoma. Predictive value of tumour-infiltrating lymphocytes. Br.J.Cancer, 85: 1871-1877, 2001.
182. Saio, M., Radoja, S., Marino, M., and Frey, A. B. Tumor-infiltrating macrophages induce apoptosis in activated CD8(+) T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide. J.Immunol., 167: 5583-5593, 2001.

183. Hansson, M., Hermodsson, S., Brune, M., Mellqvist, U. H., Naredi, P., Betten, A., Gehlsen, K. R., and Hellstrand, K. Histamine protects T cells and natural killer cells against oxidative stress. J.Interferon Cytokine Res., 19: 1135-1144, 1999.
184. Hansson, M., Asea, A., Hermodsson, S., and Hellstrand, K. Histaminergic regulation of NK-cells: protection against monocyte- induced apoptosis. Scand.J.Immunol., 44: 193-196, 1996.
185. Hellstrand, K., Asea, A., and Hermodsson, S. Histaminergic regulation of antibody-dependent cellular cytotoxicity of granulocytes, monocytes, and natural killer cells. J.Leukoc.Biol., 55: 392-397, 1994.
186. Hellstrand, K. and Hermodsson, S. Cell-to-cell mediated inhibition of natural killer cell proliferation by monocytes and its regulation by histamine H2-receptors. Scand.J.Immunol., 34: 741-752, 1991.
187. Brune, M. and Hellstrand, K. Remission maintenance therapy with histamine and interleukin-2 in acute myelogenous leukaemia. Br.J.Haematol., 92: 620-626, 1996.
188. Lurie, Y., Nevens, F., Aprosina, Z. G., Fedorova, T. A., Kalinin, A. V., Klimova, E. A., Ilan, Y., Maevskaya, M. V., Warnes, T. W., Yuschuk, N. D., Hellstrand, K., and Gehlsen, K. R. A multicentre, randomized study to evaluate the safety and efficacy of histamine dihydrochloride and interferon-alpha-2b for the treatment of chronic hepatitis C. J.Viral Hepat., 9: 346-353, 2002.
189. Mellqvist, U. H., Wallhult, E and others. Histamine dihydrochloride, interleukin-2 and interferon-alfa in multiple myeloma. Int.J.Immunother. 15, 125-130. 1999.
190. Schmidt, H., Larsen, S., Bastholt, L., Fode, K., Rytter, C., and von der Maase, H. A phase II study of outpatient subcutaneous histamine dihydrochloride, interleukin-2 and interferon-alpha in patients with metastatic melanoma. Ann.Oncol., 13: 1919-1924, 2002.
191. Agarwala, S. S., Glaspy, J., O'Day, S. J., Mitchell, M., Gutheil, J., Whitman, E., Gonzalez, R., Hersh, E., Feun, L., Belt, R., Meyskens, F., Hellstrand, K., Wood, D., Kirkwood, J. M., Gehlsen, K. R., and Naredi, P. Results From a Randomized Phase III Study Comparing Combined Treatment With Histamine Dihydrochloride Plus Interleukin-2 Versus Interleukin-2 Alone in Patients With Metastatic Melanoma. J.Clin.Oncol., 20: 125-133, 2002.
192. Agarwala, S. S., Hellstrand, K., Gehlsen, K., and Naredi, P. Immunotherapy with histamine and interleukin 2 in malignant melanoma with liver metastasis. Cancer Immunol.Immunother., 53: 840-841, 2004.
193. Donskov, F., von der Maase, H., Henriksson, R., Stierner, U., Wersall, P., Nellemann, H., Hellstrand, K., Engman, K., and Naredi, P. Outpatient treatment with subcutaneous histamine dihydrochloride in combination with interleukin-2 and interferon-alpha in patients with metastatic renal cell carcinoma: results of an open single-armed multicentre phase II study. Ann Oncol, 13: 441-449, 2002.
194. Hellstrand, K., Hansson, M., and Hermodsson, S. Adjuvant histamine in cancer immunotherapy. Semin.Cancer Biol., 10: 29-39, 2000.
195. Hellstrand, K., Brune, M., Naredi, P., Mellqvist, U. H., Hansson, M., Gehlsen, K. R., and Hermodsson, S. Histamine: a novel approach to cancer immunotherapy. Cancer Invest, 18: 347-355, 2000.
196. Hellstrand, K., Brune, M., Dahlgren, C., Hansson, M., Hermodsson, S., Lindner, P., Mellqvist, U. H., and Naredi, P. Alleviating oxidative stress in cancer immunotherapy: a role for histamine? Med.Oncol., 17: 258-269, 2000.
197. Bukowski, R. M., Negrier, S., and Elson, P. Prognostic factors in patients with advanced renal cell carcinoma: development of an international kidney cancer working group. Clin.Cancer Res., 10: 6310S-6314S, 2004.