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RENAL CELL CARCINOMA: CHANGES IN NATURAL HISTORY AND TREATMENT OF METASTATIC DISEASE

PEETER PADRIK

TARTU UNIVERSITY
PRESS

Clinic of Hematology and Oncology, Tartu University Hospital, Tartu, Estonia
Department of Hematology and Oncology, Faculty of Medicine, University of Tartu, Tartu, Estonia

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Opponent: Professor Mirja Ruutu, MD, PhD, University of Helsinki, Helsinki, Finland

Commencement: November 30, 2007

Supervisor: Professor Hele Everaus, MD, DSc (Medicine), Department of Hematology and Oncology, Faculty of Medicine, University of Tartu, Tartu, Estonia

Reviewers: Professor Tiit Haviko, MD, DSc (Medicine), Department of Traumatology and Orthopaedics, University of Tartu, Tartu, Estonia
Professor Margus Lember, MD, PhD, Department of Internal Medicine, University of Tartu, Tartu, Estonia

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Dedicated to all patients suffering from this disastrous disease

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the publications below, which will be referred to in the following by their Roman numerals:

I. Padrik P, Kirsimagi U, Everaus H. Changes in the natural history of renal cancer: comparison of Estonian data from the periods 1986–1988 and 1996–1998. Int Urol Nephrol 2007; 39 (1):35-41. Epub 2006 Oct 25.
II. Padrik P. Prognostic factors of immunotherapy in metastatic renal cell carcinoma. Med Oncol 2003; 20 (4):325–34.
III. Padrik P, Leppik K, Arak A. Combination therapy with capecitabine and interferon alfa-2A in patients with advanced renal cell carcinoma: a phase II study. Urol Oncol 2004; 22 (5):387–92.
IV. Padrik P, Saar H, Leppik K. Application of analysis of thymidine phosphorylase expression in metastatic renal carcinoma tissue. Estonian Physician 2006; 85 (4):297–300 (in Estonian, summary in English).

ABBREVIATIONS

CI – confidence interval
CT – computed tomography
ECOG – Eastern Cooperative Oncology Group
HR – hazard ratio
IFNa – interferon-alfa
IFNa2A – interferon-alfa2A
IL-2 – interleukin-2
NCI CTC – National Cancer Institute Common Toxicity Criteria
RCC – renal cell carcinoma
TP – thymidine phosphorylase
U – units
US – ultrasonography

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1. INTRODUCTION

There are an estimated 190,000 new renal cancer cases and 91,000 deaths from renal cancer in the world each year (Parkin et al. 2001). New renal cancer cases constitute 1.9% of all cancer cases and deaths constitute 1.5% from all cancer deaths (Parkin et al. 1999). Renal cancer has the highest rates in North America and in Western, Eastern, and Northern Europe. Incidence rates are low in Africa, Asia (except in Japanese males), and the Pacific (Parkin et al. 1999).

The most common malignant lesion of the kidney is renal cell carcinoma (RCC), accounting for approximately 85% of all renal cancers. Nephroblastoma (i.e., Wilms tumor) accounts for 5% to 6% and transitional cell neoplasm of the renal pelvis for 7% to 8%; the remainder are various sarcomas of renal origin (Richie et al. 2003). The main histopathologic types of RCC are clear cell, papillary, and chromophobe carcinoma (Eble et al. 2004).

If the disease is confined to the kidney, surgical resection is the treatment of choice and often recovery can result (Pantuck et al. 2001; Vogelzang & Stadler 1998). Unfortunately, approximately 20–30% of patients with RCC present with metastases and another 20–40% of patients undergoing nephrectomy for clinically localized disease will ultimately develop metastases (Lam et al. 2005). Frequent metastatic sites include the lung parenchyma (in 50–60% of patients with metastases), bone (in 30–40%), liver (in 30–40%), and brain (in 5%) (Motzer et al. 1996). Unusual sites of metastases are characteristic of RCC, and virtually any organ site can be involved, including the thyroid, pancreas, skeletal muscles, and skin or underlying soft tissue.

For metastatic disease, systemic medical therapy is necessary. For a long time, the only available therapy has been immunotherapy with interleukin-2 (IL-2) or interferon-alfa (IFNa), but the efficacy of these treatments has been quite limited. Because of the overall poor results with drug treatment, patients with metastatic RCC should be considered for clinical trials, especially for phase I and II trials evaluating newer agents.

According to the Estonian Cancer Registry's latest data from the year 2003, renal cancer is the 8th most common malignancy among men in Estonia (130 new cases, age-standardized incidence 15.3 per 100,000) and 10th among women (103 new cases, age-standardized incidence 6.8 per 100,000) (Estonian Cancer Registry 2006). In the past, survival rates of renal cancer have been very low in Estonia, but improved remarkably during the 1990s (Damhuis & Kirkels 1998).

The current dissertation treats different aspects of RCC management: changes in diagnosis and surgical treatment, prognostic factors of immunotherapy and combination therapy in metastatic disease with IFNa and capecitabine. All these different aspects are important for the final outcome of the therapy of this serious disease.

2. REVIEW OF THE LITERATURE

2.1. Changes in the natural history of renal cancer

The incidence of and mortality from RCC have continuously increased during the last 50 years (Pantuck et al. 2001). Despite this increase in the number of new patients and consequently in the number of deaths yearly, the percentage of those surviving for 5 years has improved noticeably, patients with localized and metastatic renal cancer have had improvements in their outlook and the therapeutic options available have expanded (Pantuck et al. 2001).

The collaborative EUROCARE II study showed that there has been a time trend in survival of renal carcinoma patients in Europe: age-standardized 5-year survival improved from 44% in 1978–1980 to 50% in 1987–1989 (Damhuis & Kirkels 1998). The European 5-year relative survival rate for renal cancer patients diagnosed during the period 1985–1989 was 48%, but large variations were observed between countries with 5-year relative survival ranging from 30% to 57% (Damhuis & Kirkels 1998). In the EUROCARE III study, the European 5-year relative survival rate for patients diagnosed in 1990–1994 was 57%, the lowest and the highest survival rates ranging 34–67% for men and 35–68% for women (Sant et al. 2003).

A steady increase in the incidence of renal cancer has been observed in Estonia since the late 1960s and has continued during recent years. Age-standardized incidence increased from 6.0 to 17.2 per 100,000 for men and from 2.2 to 7.2 per 100,000 for women in 1968–2000 (Aareleid & Rahu 1991; Aareleid & Mägi 1999; Aareleid & Mägi 2000; Aareleid & Mägi 2001). Survival rates for Estonian patients have been among the lowest in EUROCARE studies: the 5-year relative survival rate for men and for women was 30% in the period 1985–1989 and 34% for men and 53% for women in 1990–1994 (Damhuis & Kirkels 1998; Sant et al. 2003). Nevertheless considerable changes have taken place; the 5-year relative survival rate increased from about 25% to more than 50% (Aareleid & Brenner 2002). Although the prognosis for patients with this malignancy has also improved over time in other countries, the degree of the improvement in Estonia has been remarkable (Aareleid & Brenner 2002; Damhuis & Kirkels 1998).

The most important determinant of prognosis for a patient with primary RCC is the pathologic stage in which the tumor is initially diagnosed and treated (size and extent of the primary tumor, status of regional lymph nodes, and presence of distant metastases); the other important factors are tumor grade and the patient‘s performance status (Lam et al. 2005; Pantuck et al. 2001). It has been proposed that variation in survival rates according to the country or time is probably related to the differences in the distribution of tumor stage at diagnosis (Aareleid & Brenner 2002; Damhuis & Kirkels 1998). The increase in

the number of earlier cases may be mainly due to improved diagnostic techniques, which are able to detect small tumors (Pantuck et al. 2001). However, there is no direct study to prove that. Therefore, we performed the analysis to find the factors which influenced the increase of RCC survival rates in Estonia in 1990s.

Radical nephrectomy (removal of the kidney, perinephric fat, adjacent lymph nodes, and often the adrenal gland) is currently the main therapy for RCC (Mickisch et al. 2001a; Richie & Kantoff 2003; Stewart & Kleihues 2003). Radical nephrectomy is accomplished by early ligation of the renal artery, renal vein, and en bloc removal of the kidney with the surrounding Gerota's fascia. This procedure has been shown to produce better survival rates than simple nephrectomy (kidney removal only), since involvement of regional lymphatic and periaortic lymph nodes has been noted in almost 25% of the patients (Richie & Kantoff 2003; Stewart & Kleihues 2003). The concept of radical nephrectomy with en bloc excision of Gerota's envelope was advocated by Robson and colleagues, who reported a 5-year survival rate of 66% compared to the previous cumulative surgical rates of 48% for simple nephrectomy (Robson et al. 1968). In cases of small (<4 cm) peripheral lesions, an organ-sparing approach may be considered. Emerging data also suggest that partial nephrectomy can also be performed on tumors larger than 4 cm (Lam et al. 2006). Changes in surgical techniques may be one of the factors behind the improvement of survival rates of RCC patients.

A formal lymph node dissection is a valuable diagnostic tool for staging; however, its therapeutic efficacy is has not been proved. As regional lymphadenectomy adds little in terms of operative time or risk, it should be included in conjunction with radical nephrectomy (Lam et al. 2006; Mickisch et al. 2001a; Richie & Kantoff 2003).

If surgery cannot eradicate all tumor deposits, nephrectomy remains palliative therapy and should be considered in the context of multimodality therapy (e.g. in conjunction with immunotherapy) (Mickisch et al. 2001a). Clinical situations in which surgery is potentially appropriate include excision of locally recurrent disease, excision of solitary or multiple metastasis, resection of a residual mass after systemic therapy, and palliation (Lam et al. 2006).

Advances in medical therapy for metastatic disease have also influenced the natural history of RCC, see section 2.3. Medical therapy for metastatic RCC.

In conclusion, the improved survival has been associated with advances in renal imaging, earlier diagnosis, improved staging, better understanding of prognostic indicators, refinement in surgical techniques, and the introduction of immunotherapy approaches for advanced disease (Pantuck et al. 2001).

2.2. Prognostic factors of metastatic renal cell carcinoma

Prognostic factors of survival or therapy in metastatic RCC can be used in clinical management as well as in clinical trial design and interpretation. Until better treatments are available, the aim of systemic therapy in metastatic RCC is to prolong survival without having a negative impact on the quality of life. Therefore, it is appropriate to try to identify patient factors that can predict long survival times and/or responsiveness to immune therapies. The survival of patients with metastatic RCC is related to the intrinsic biological characteristics of the individual patient's malignancy, response proportions to INFa, IL-2 or combination programs vary considerably among clinical trials (Bukowski 2000; Bukowski 2001; Coppin et al. 2000; Fossa 2000). Thus, patient selection is an important factor in achieving a favorable treatment outcome as only a small minority of patients experience a therapeutic benefit, whereas most patients suffer from the important adverse effects of the treatments. According to the latest studies, the French Immunotherapy Intergroup's PERCY Quattro trial showed no significant improvement in survival with the use of cytokines, alone or in combination, when compared with a medroxyprogesterone acetate control in patients with an intermediate prognosis for response to cytokine treatment (Negrier et al. 2005).

The impacts of different factors for prognosis in metastatic RCC have been analyzed in several non-immunotherapy (chemotherapy and hormone therapy or heterogeneous treatment modalities) studies. Data about these studies are presented in Publication II. It can be concluded from these analyses that better prognosis for survival in metastatic RCC is associated with a normal performance status, longer disease-free period, limited number of metastatic sites, and normal laboratory characteristics.

Studies analyzing prognostic factors in multivariate analyses in patients with metastatic RCC treated with various immunotherapy regimens are characterized in Table 1, Publication II. In different studies independent prognostic values according to multivariate analyses were detected for: performance status, time interval from diagnosis to treatment of metastatic disease, time interval from renal tumor to metastases, number of metastatic sites, prior nephrectomy, weight loss, sarcomatoid histology, bone metastases, liver metastases, extrapulmonary metastases, pretreatment erythrocyte sedimentation rate, elevated lactate dehydrogenase, elevated neutrophil count, low serum hemoglobin, high corrected serum calcium, biological signs of inflammation, elevated level of alkaline phosphatase, elevated C-reactive protein, nephrectomy (Publication II). In several immunotherapy studies patients were divided into prognostic groups (see Table 3, Publication II). Mostly prognostic subgroups of patients appear as a function of the number of risk factors present. Each factor, as reported in the literature, is usually associated with a rather limited increase in the risk of death, since most relative risks are between 1 and 2. Because each

study has analyzed different parameters and none of these factors have been associated with a very high relative risk of death, risk stratification groups often differ from one model to another. Therefore, a comparative analysis was performed about all prognostic factors which have been studied in the literature and those which have demonstrated an independent prognostic significance in multivariate analyses.

2.3. Medical therapy for metastatic renal cell carcinoma

2.3.1. Chemotherapy

RCC is mostly a chemotherapy-resistant tumor, which exhibits only marginal response rates; no clear survival benefit for patients treated with chemotherapy has yet been demonstrated (Amato 2000; Hartmann & Bokemeyer 1999; Motzer 2000; Yagoda et al. 1995). Yagoda et al. have analyzed the data from the phase II clinical trials conducted between 1983 and 1993 with more than 4500 patients. The analysis showed that the mean objective response to treatment in more than 70 different medications was 6.8% (Yagoda et al. 1995). Motzer has done summarizing analysis of the results of 51 phase II clinical trials with 1347 patients, conducted between 1990 and 1998. The most frequently studied medications were floxuridine and fluorouracil with treatment response of 0–20%. The treatment responses were also generally of short duration (Motzer 2000). It can be concluded that so far no chemotherapeutic drug has proved such an anticarcinogenic effect that would warrant its use as standard treatment in RCC.

Among newer medicines, the effectiveness of capecitabine has been evaluated. Capecitabine is a relatively new orally administered prodrug that is selectively tumor-activated to its cytotoxic moiety, 5-fluorouracil, by thymidine phosphorylase (TP). Capecitabine is capable of exploiting the high concentrations of TP in tumor tissue to achieve activation preferentially at the tumor site (Ishikawa et al. 1998; Mackean et al. 1998; Miwa et al. 1998; Schuller et al. 2000). Tumor types known to have a high level of TP activity, such as RCC, are especially attractive targets for capecitabine therapy (Twelves 2001). Capecitabine has potential as a combination partner for IFNa immunotherapy with different mechanisms of action, possible synergism and little overlapping of key toxicities. The combination of capecitabine with subcutaneous recombinant human IFNa, recombinant human IL-2, and oral 13-cis-retinoic acid has shown promising efficacy in the first-line treatment of metastatic RCC (Oevermann et al. 2000), also as monotherapy after immunotherapy failure (Wenzel et al. 2002). A phase I study of capecitabine and IFNa has demonstrated moderate toxicity with recommended dosage levels for phase II trials 3.0

million units (U)/m² subcutaneously three times weekly for IFNa and 1,000 mg/m² twice daily for 2 weeks for oral capecitabine (Chang et al. 2001).

2.3.2. Hormone therapy

Hormonal agents have been tested in RCC treatment for several decades. RCC tissue contains estrogen, progesterone and testosterone receptors. The first promising results with progesterone led to further studies with tamoxifen or toremifen which had the objective treatment response in 17% of patients (Schomburg et al. 1993; Stahl et al. 1992). The treatment responses were of short duration and no medication had a positive effect on life expectancy. Hormonal therapy with tamoxifen and medroxyprogesterone has been used as the best supportive treatment in the medical practice of many countries; however, it cannot be recommended.

2.3.3. Immunotherapy

Until recently the most promising systemic therapy for metastatic RCC has been immunotherapy, and although there has been no internationally recognized standard therapy, patients have been treated with INFa or IL-2 monotherapy, or combinations outside of clinical trials.

In 2001, the analysis of metastatic RCC immunotherapy by Coppin et al. (Cochrane Collaboration) was published. The data of 42 randomized clinical trials with 4216 patients were analyzed; the survival analysis was based on 26 randomized trials with 3089 patients. The average response rate was 10.2% (0–39%) and the complete response rate was 3.2% (123 cases out of 3852; n=38 studies). The median survival rate was 11.6 months (6–28 months) and the median two-year survival was 22% (16 studies, 8–41%) (Coppin et al. 2000). In 2005, a new extensive systematic review was published, where combined data for a variety of immunotherapies gave an overall chance of partial or complete remission of 12.9% (99 study arms), compared to 2.5% in 10 non-immunotherapy control arms, and 4.3% in two placebo arms (Coppin et al. 2005). 28% of these remissions were designated as complete (data from 45 studies). Median survival averaged 13.3 months.

Interferons are glycoproteins found in the human body. They have antiviral, immunomodulatory and antiproliferative features. Three classes of interferons have been identified: IFNa, interferon-beta and interferon-gamma. Only a few patients with RCC have been treated with interferon-beta. Interferon-gamma has not shown anticarcinogenic activity against RCC in a randomized double-blind placebo-controlled clinical trial (Gleave et al. 1998). However, IFNa is widely used in RCC therapy.

The potential role of IFNa in improvement of survival compared to other treatment modalities has been studied in several randomized clinical trials. No statistically significant differences have been found in studies with a limited number of patients, but larger studies have proved that treatment with IFNa improves survival rates. Studies reported by Pyrhonen et al., with 160 patients (Pyrhonen et al. 1999) and the Medical Research Council Collaborators, with 335 patients (Medical Research Council Renal Cancer Collaborators 1999), showed that INFa significantly enhances survival, it was superior to treatment with hormones only and its addition to vinblastine was beneficial. Pyrhonen et al. investigated in their randomized trial the effects of IFNa and vinblastine combination therapy compared to vinblastine monotherapy. Response to therapy was 16.5% for combination and 2.5% for monotherapy, the median survival rates were 15.8 and 8.8 months respectively. The study showed that IFNa can possibly improve patient survival, because the effect of vinblastine monotherapy was minimal. The randomized trial by the Medical Research Council Collaborators compared subcutaneously administrated IFNa and medroxyprogesterone acetate. With IFNa, the 1-year survival was improved by 12% (43% versus 31%) and the median survival rate by 2.5 months (8.5 versus 6 months).

Extensive review of the available data by the Cochrane Collaboration (Coppin et al. 2005) identified four randomized controlled studies (Medical Research Council Renal Cancer Collaborators 1999; Kriegmair et al. 1995; Pyrhonen et al. 1999; Steineck et al. 1990), involving 644 patients, in which IFNa was compared directly with noncytokine treatment. Pooled analysis showed a remission rate of 40 (12.5%) of 320 for IFNa versus 5 (1.5%) of 324 for controls. IFNa was associated with reduced 1-year mortality, odds ratio for death by 1 year 0.56; 95% confidence interval (CI), 0.40 to 0.77. Similarly, the pooled overall hazard ratio (HR) for death in the studies (644 patients) showed a survival advantage for INFa (HR, 0.74; 95% CI, 0.63 to 0.88) (Coppin et al. 2005).

The optimal dose of IFNa has not yet been defined, but 5–20 million U of recombinant IFNa a day is considered effective (Motzer 2000). High doses of IFNa can cause unacceptable toxicity and based on phase II and III clinical studies, the standard monotherapy dose is 3–10 million U/m^2 of IFNa administered via subcutaneous or intramuscular injections 3 to 5 days a week (Fossa 2000). The optimal duration of IFNa treatment has not been determined, but the majority of researchers recommend at least 3 months of therapy and in case of a good effect, it can be extended to 1 year (Medical Research Council Renal Cancer Collaborators 1999). Frequently occurring side effects at the beginning of IFNa therapy are fever, myalgia and asthenia (flu-like symptoms).

It can be concluded that IFNa offers a moderate increase in survival compared to other modalities of treatment for patients with metastatic RCC, and it has been considered as the standard systemic therapy option for metastatic

RCC. Moreover, it has been considered as a control branch in further randomized trials (Coppin et al. 2005; Fossa 2000).

IFNa combination with chemotherapy. IFNa combination with vinblastine has been studied in phase II trials and the treatment response has been from 11% to 43%, mean 20% (Pittman et al. 1994). Two of these phase II trials did not show the improvement of survival with this combination compared to IFNa monotherapy (Fossa et al. 1992; Neidhart et al. 1991). IFNa has also been combined with 5-fluorouracil, but the toxicity was high and the treatment effects did not improve (Haarstad et al. 1994).

Based on synergism between IFNa and retinoids found *in vitro* and *in vivo* studies, three phase III trials have examined the effect of the addition of 13-cis-retinoic acid to IFNa; one of them reported prolongation of progression-free and overall survival, but other studies failed to show survival advantage (Aass et al. 2005; Atzpodien et al. 2004; Motzer et al. 2000).

Interleukin-2. Marked progress in immunotherapy has been achieved with the identification of cytokine IL-2. IL-2 is a growth factor that enhances the proliferation and function of lymphocytes and plays an important role in the regulation of immune reactions. Although IL-2 does not have a direct cytotoxic effect on tumor cells, it can cause tumor regression by stimulating the cellular immune response *in vivo*. The IL-2 anticarcinogenic effect is related to the ability to dilate and activate subtypes of cytotoxic lymphocytes that express IL-2 receptors (Margolin 2000). When administered in pharmacological doses to patients with intact organ functions and good health condition, IL-2 has induced a stable complete therapeutic effect in 5% and partial effect in 10–15% of patients (Margolin 2000).

The use of IL-2 as medication was established based on a multicentric clinical study including 255 patients: high intravenous bolus doses of IL-2 (600,000 or 720,000 U/kg) were used and objective therapeutic effect of 15% (7% complete and 8% partial) was observed. The drug-related mortality was 4%. The mean duration of the therapeutic effect in all patients who positively reacted to treatment has been 84 months (Fyfe et al. 1995). Complete therapeutic responses have often been stable and even effects lasting more than 130 months have been observed.

The metastatic kidney cancer immunotherapy analysis performed by Coppin et al. stated that no randomized placebo-controlled and survival trials have been conducted with high-dose IL-2 (Coppin et al. 2005; Coppin et al. 2000). It can be concluded that systemic treatment with IL-2 is beneficial to those patients who react to treatment, but since they are the minority, other treatment possibilities are needed for the majority of patients.

IL-2 and IFNa combinations. Preclinical studies of different tumor models have provided data indicating the possible synergistic effect of IL-2 and IFNa (Chikkala et al. 1990). Therefore, numerous clinical trials have been carried out with the combination of IL-2 and IFNa, using different doses, schemes and ways of administration. The therapeutic response to this cytokine combination has been about 20% (Bukowski 2000). At that the responses do not depend on the different ways of IL-2 administration. Total disease regression has been observed in 3 to 5% of patients (Bukowski 2000). Analysis by Coppin et al. indicated that IL-2 therapeutic value has not been proven in randomized trials and therefore adding IL-2 to IFNa therapy is not recommended outside phase III clinical trials (Coppin et al. 2000). Thus, clinical trials have not proven that IL-2 and IFNa combination has advantages over respective monotherapies; only one study has shown a modest superiority of IL-2 and IFNa combination over monotherapy with a better treatment response and 1-year survival rate (Negrier et al. 1998; Negrier et al. 2000; Negrier et al. 2005).

IFNa and IL-2 combinations with 5-fluorouracil have also been studied in several clinical trials with different schemes and contradicting results have been obtained. Some studies have shown high and others very low treatment responses, ranging from 1.8 to 39%; mean 25.3% (n=836). The mean survival rate also varies (Bukowski 2000). This shows that patient selection and prognostic factors could play an important role in these studies.

Immunotherapy in combination with surgery

The treatment results of IFNa and IL-2 are influenced directly by several risk factors associated with the patient's condition and disease stage. Tumor nephrectomy done before immunotherapy has been considered a factor for better prognosis. Such cytoreductive management has so far been studied in two randomized phase III trials where the effects of two treatment schemes were compared: nephrectomy followed by IFNa treatment or IFNa alone. Both studies proved the preference of combination therapy with the improvement in the patient survival rate (in the Southwest Oncology Group Study 12.5 versus 8.5 months; in the European Organization for Research and Treatment of Cancer Study 17 versus 7 months) (Flanigan et al. 2001; Mickisch et al. 2001b). Therefore, palliative cytoreductive tumor nephrectomy must be considered standard therapy in the context of metastatic RCC immunotherapy (in patients who can undergo the operation).

Other forms of immunotherapy currently under research for the treatment of RCC are vaccines, antibodies directed at immune response modulators, immune-cell transfer, and allotransplantation (Yang & Childs 2006).

2.3.4. New targeted therapies

A number of small molecules targeted at the tyrosine kinase domains of a variety of growth factor receptors have now been evaluated in clinical trials. Currently two kinase inhibitors – sorafenib and sunitinib – are approved for the treatment of patients with metastatic RCC.

Sorafenib has demonstrated a 39% improvement in survival compared with the placebo in second-line therapy after one prior immunotherapy (Escudier et al. 2005). The median progression free survival in the sorafenib arm was 5.5 months compared with 2.8 months in patients receiving placebo, the median survival was 19.3 months for sorafenib versus 14.3 months for placebo (Eisen et al. 2006).

An international phase III randomized trial compared sunitinib versus INFa as first-line systemic therapy for patients with metastatic RCC (Motzer et al. 2006). The median progression-free survival was 47.3 weeks for sunitinib versus 24.9 weeks for IFNa. The objective response rate was 24.8% for sunitinib versus 4.9% for IFNa. These results demonstrated a statistically significant improvement in progression free survival and objective response rate for sunitinib over IFNa in first-line treatment of patients with metastatic RCC.

Other promising new agents in clinical trials are CCI-779 or temsirolimus (Atkins et al. 2004; Hudes et al. 2006) and bevazicumab (Yang et al. 2003).

Despite the availability of these new agents, early entry of patients into clinical trials is warranted. The optimal use of new agents is still the subject of intense clinical research interest. In this context we performed a phase II study to evaluate the efficacy and safety of the capecitabine and IFNa2A combination as the first line treatment in patients with metastatic RCC (Publication III).

2.4. Thymidine phosphorylase expression in tumor tissue

For a better treatment optimization, predictive factors of therapy are needed. TP is one possible candidate for the prediction of capecitabine efficacy. TP is the enzyme that metabolizes 5'-deoxy-5-fluorouridine, an intermediate metabolite of capecitabine, to the active drug 5-fluorouracil. Capecitabine is a fluoropyrimidine carbamate capable of exploiting the high concentrations of TP in tumor tissue to achieve activation preferentially at the tumor site. Measurements of TP concentrations in tumor tissues may provide an indicator of increased sensitivity to capecitabine. Studies by Imazano et al., Mizutani et al., and Morita et al. have demonstrated that the enzymatic activity of TP is higher in RCC tissue than in normal kidney tissue (Imazano et al. 1997; Mizutani et al. 2003; Morita et al. 2003). Hirano et al. and Ikemoto et al. have demonstrated in vitro studies a significant positive correlation between the TP activity and

5-fluorouracil sensitivity, suggesting that TP may be useful as a predictive factor in combination therapy with interferon gamma and 5-fluorouracil or 5'-deoxy-5-fluorouridine (Hirano et al. 2003; Ikemoto et al. 2002). In vitro study by Morita et al. investigated the modulatory actions of human recombinant interferon-alfa2A (INFa2A) on 5-fluorouracil in five RCC cell lines in vitro, in particular focusing on TP expression (Morita et al. 1999). Results of the study suggested that INFa upregulates TP expression and modulates 5-fluorouracil anabolism, thus enhancing 5-fluorouracil cytotoxicity in a dose- and schedule-dependent manner in some RCC cells. The results also implied that TP expression measurement in RCC could identify subgroups of metastatic RCC, which may respond to INFa/5-fluorouracil combination therapy, and sequential administration of INFa followed by 5-fluorouracil may be beneficial in such cases. Another in vitro study by Morita et al. provided direct evidence that TP has the role in mediating the sensitivity of RCC to capecitabine (Morita et al. 2001).

So far TP expression measurement is not used in everyday clinical practice as it's clinical value is unclear. Therefore, we implemented TP expression analysis in RCC tissue with immunohistochemistry assays with monoclonal anti-TP antibody to evaluate level of TP expression and possible correlation between TP expression values and the treatment efficacy of capecitabine and INFa2A combination (Publication IV).

3. AIMS OF THE STUDY

The aims of the present study were:

1. To explain the differences in survival of RCC patients in Estonia in the 1980s and 1990s, comparing the stage distribution of RCC and the diagnostic and treatment methods used (Publication I).

2. To find out the most consistent prognostic factors of survival in patients receiving immunotherapy in metastatic RCC (Publication II).

3. To evaluate the efficacy and safety of the capecitabine and IFNa2A combination as the first line treatment in patients with metastatic RCC (Publication III).

4. To evaluate the level of TP expression in RCC tissue with immunohistochemistry assays with monoclonal anti-TP antibody and possible correlation between TP expression values and treatment efficacy of capecitabine and INFa2A combination (Publication IV).

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4. PATIENTS AND METHODS

The thesis is based on three studies and an analysis of literature. The main characteristics of the studies are presented in Table 1.

Table 1. The characteristics of the 4 studies constituting the thesis.

Study number	Study type	Study site	Study period	Number of patients	Goals of the study	Publication number
1.	Retrospective	Tartu Maarjamõisa Hospital; Tartu Oncology Hospital; Estonian Cancer Centre	1986–1988 and 1996–1998	427	Changes in diagnostics, treatment and survival	Publication I
2.	Literature analysis	9 international studies	1982–1998	2417	Prognostic factors of immunotherapy	Publication II
3.	Prospective phase II trial*	Tartu University Hospital	2001–2003	25	Combination therapy with INFa and capecitabine	Publication III
4.	Prospective*	Tartu University Hospital	2001–2003	16	TP expression analysis	Publication IV

* Same database of patients.

4.1. Evaluation of the natural history of renal cancer in Estonia

We performed a retrospective analysis based on hospital files for all primary inpatients with RCC diagnosed in 1986–1988 and 1996–1998 in three different Estonian hospitals (Tartu Maarjamõisa Hospital, Tartu Oncology Hospital and Estonian Cancer Centre in Tallinn). Patients with tumors of the renal pelvis, ureter, and nephroblastomas were not included. The data of age, sex, stage and TNM status, renal vein invasion, grade of the tumor, histological type, diagnostic methods, operation type, and radio- and systemic therapy were collected. Comparative analysis was performed for the both periods regarding stage distribution, diagnostic methods, and operation type. Using data from operation and pathology reports, performed nephrectomies were divided into two groups: radical nephrectomies (performed according to a standard technique of radical nephrectomy) and simple nephrectomies (not performed according to the radical nephrectomy technique, performed mainly through lumbotomy approach).

4.2. Prognostic factors analysis

The literature search was performed using Medline and PubMed databases to find all metastatic RCC immunotherapy studies, which used multivariate analysis to identify independent prognostic factors. Using data from these studies, a comparison was made between all the prognostic factors analyzed and those which have demonstrated an independent prognostic significance in multivariate analyses. Only studies where treatment contained immunotherapy were focused on for this comparison. All prognostic factors were divided into different groups to draw together similar factors.

4.3. Patients and methods in phase II clinical trial with capecitabine and INFa2A

The eligibility criteria included being of age 18–75 years; informed consent; histologically confirmed locally advanced (unresectable primary tumor) or metastatic (M1 according to TNM classification) RCC; no prior chemotherapy, immunotherapy or hormonal therapy, or radiotherapy to the measurable lesion site; measurable disease (at least one unidimensionally measurable lesion with at least one diameter ≥2 cm, as assessed by physical or X-ray examination or at least ≥1 cm as assessed by spiral computed tomography (CT) scan); performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) performance status scale and adequate hematological, renal, and hepatic function. The exclusion criteria included in addition presence of central nervous system metastases; concurrent pregnancy or lactation period; patients of child-bearing potential unwilling to use effective means of contraception; previous or concurrent malignancy, except cured skin basalioma or in situ carcinoma of the cervix; concurrent cardiac insufficiency or cardiac pathology (documented cardiac infarction within previous 8 weeks or medically nonresponsive hypertension); concurrent infection. The local ethics committee approved the protocol. All patients gave written informed consent before any study-specific procedures were performed.

Capecitabine (Xeloda; Hoffmann-La Roche Ltd., Basel, Switzerland) was administered orally at a dose of 1,250 mg/m² twice daily for 14 days followed by a 7-day rest period, IFNa2A (Roferon A; Hoffmann-La Roche Ltd., Basel, Switzerland) was administered subcutaneously at 6 million U three times weekly. The treatment cycle was 3 weeks long. Treatment was continued for 12 cycles or until disease progression, unacceptable toxicity, or if the patient chose to discontinue treatment. On baseline physical examination, complete blood count, serum chemistry, electrocardiography, and tumor measurements were performed. Patients were monitored before each cycle of therapy in 3-week

intervals with physical examination, complete blood count, and serum chemistry.

Tumor measurements were performed by CT-scan, chest X-ray, or physical examination at 6-week intervals (after every two cycles of treatment) until the tumor progressed. Tumor response was classified on the basis of the response evaluation criteria in solid tumor (RECIST) guidelines (Therasse et al. 2000).

Adverse events were monitored continuously during treatment and for 28 days after the last dose of therapy. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 2.0. For hand-foot syndrome, the following grading system was used: grade 1 hand-foot syndrome is defined as numbness, dysesthesia/paresthesia, tingling, painless swelling or erythema of the hands and/or feet and/or discomfort which does not disrupt the patient's normal activities; grade 2 hand-foot syndrome is painful erythema and swelling of the hands and/or feet and/or discomfort affecting the patient's activities of daily living; grade 3 hand-foot syndrome is moist desquamation, ulceration, blistering and severe pain of the hands and/or feet and/or severe discomfort that causes the patient to be unable to work or perform activities of daily living.

Dose modification principles and methods of statistical analysis are described in Publication III.

4.4. Methods of thymidine phosphorylase expression analysis

TP expression was evaluated retrospectively with immunohistochemistry assays using monoclonal anti-TP antibody (Roche Diagnostics GmbH) in formalin-fixed, paraffin-embedded specimens. Signed informed consents and paraffin-embedded specimens were for that purpose available from 16 patients treated with INFa and capecitabine combination.

A semi-quantitative analysis using a scoring system for tumor cells was performed, where scores were calculated as sum of staining percentage and staining intensity scores, ranging from 0 to 7:

Staining pattern of TP in tumor cells	**Score**
No staining is observed in tumor cells	0
Staining is observed in 0–10% of the tumor cells	1+
Staining is observed in 10–25% of the tumor cells	2+
Staining is observed in 25–50% of the tumor cells	3+
Staining is observed in 50–100% of the tumor cells	4+

Staining intensity of TP in tumor cells	**Score**
No staining is observed in tumor cells	0
TP expressing tumor cells exhibit a weak staining intensity	1+
TP expressing tumor cells exhibit a strong staining intensity	2+
TP expressing tumor cells exhibit a very strong staining intensity	3+

5. RESULTS

5.1. Changes in the natural history of renal cancer

Demographic data

Data were available for 122 patients with renal cancer diagnosed in the period of 1986–1988 and for 305 patients in the period of 1996–1998. Comparison of the patients' distribution by age, sex, and stage is presented in Table 1, Publication I. There was a statistically significant difference ($p<0.0001$) in stage distribution with an increase for stages I and II and a decrease for stages III and IV in the period of 1996–1998 in comparison with 1986–1988.

Diagnosis

Comparison of the patients' distribution by primary diagnostic methods and characteristics of morphological diagnosis are presented in Table 2, Publication I. The most frequent diagnostic method that primarily confirmed the diagnosis of renal cancer in the period of 1986–1988 was intravenous urography followed by renal angiography and abdominal ultrasonography (US), CT was not used. In the period 1996–1998 the predominating primary diagnostic method was already abdominal US; CT was used in 81.3% of patients, yielding the diagnosis primarily in 10.8% of cases.

Treatment

Comparison of operation rates and operation types is presented in Table 3, Publication I. In the period of 1986–1988 98% of patients in stages I–III and 33.8% in stage IV were operated for primary tumor removal, in the period of 1996–1998 respective figures were 96.8% and 57.8%. The increase in total operation rate in the later period was due to the higher operation rate among stage IV patients. In 1996–1998 significantly more nephrectomies were performed by the radical technique compared to 1986–1988 ($p<0.0001$), and kidney resections were also employed for smaller tumors.

Owing to the small number of patients who received radiotherapy or medical therapy, comparative analysis of these treatments was not appropriate.

Survival

Data for 1-year, 5-year and median overall survival are presented in Table 4, Publication I. These data confirm that survival improved significantly between study periods, 5-year survival being 23.8% and 47.2% and median survival being 12.0 and 48.0 months, respectively ($p<0.0001$). Overall survival was higher in the period of 1996–1998 compared with 1986–1988 both for men and women, and also in age groups ≥65 years and <65 years.

Log rank test revealed that survival data were different between stages in both periods ($p<0.0001$), but detailed analysis showed no differences in survival between stages I–III in the period of 1986–1988. Survival was significantly lower only among patients with stage IV disease. In the period of 1996–1998 survival was similar in stages I and II, but lower in stages III and IV. Survival of patients with stage I and II disease was significantly better in the later period, but there was no statistical difference between the periods in stages III and IV.

Analysis of the survival data between sexes for the periods shows statistically higher survival for women for the period of 1986–1988 (median survival 21.6 versus 9.6 months for men, $p=0.012$) and a similar trend without a statistical difference for the period of 1996–1998 (median survival 64.8 versus 43.2 months for men, $p=0.175$).

We analyzed separately survival among operated versus non-operated patients for stage IV disease. The survival of the operated patients with stage IV disease in comparison with the non-operated patients was significantly higher for later (median survival 10.6 versus 4.8 months, $p=0.002$), but not for earlier period (median survival 7.5 versus 4.6 months, $p=0.314$). However, there was no statistical difference in this characteristic between the study periods.

In univariate analysis all available factors affecting the patients' survival (time period, age, sex, stage, and operation status) proved significant (see Table 5, Publication I). The relative risk of death for patients treated in the period of 1996–1998 was 0.44 compared to those who were treated in the period of 1986–1988 ($p<0.0001$). After including all confounding covariates in the multivariate Cox model, the independent prognostic factors for overall survival were age, stage, and operation status (operated versus non-operated), the time period proved insignificant (see Table 5, Publication I). Disease stage was the strongest risk factor for patient death: compared with stage I disease, the risk for death was 6.5 times higher for the patients with stage IV disease and 2.2 times higher for stage III disease. Another significant risk factor was being operated or non-operated in stage IV disease ($RR=0.53$, $p<0.0001$), operated status was associated with a 47% reduction in the risk of death. As only a few patients with stage I–III disease were not operated, this factor is applicable only to patients with stage IV disease.

5.2. Results of the prognostic factors analysis

Analyzed prognostic factors are divided into different groups with comparison between all analyzed factors and factors which have demonstrated independent prognostic significance in multivariate analyses (Table 2, Publication II).

General patients' characteristics
From general patients' characteristics only performance status has been the independent prognostic factor in multivariate analyses, being one of the most substantial factors and showing a statistically prognostic value in seven studies out of eight.

Symptoms
The value of recent weight loss as a prognostic factor has been analyzed in 5 studies, but it has been the independent prognostic factor only in one of them (Jones et al. 1993). Lissoni et al. also analyzed the presence of anxiety, which appeared to be one of the independent prognostic factors (Lissoni et al. 1994), but the number of patients was small and the estimated value of anxiety could be problematic in larger groups of patients or outside of clinical trials.

Time characteristics
One group of investigated prognostic factors has been time periods from a renal tumor to the occurrence of metastases (Atzpodien et al. 2003; Lissoni et al. 1994; Mani et al. 1995; Negrier et al. 2002; Palmer et al. 1992), or the time from initial diagnosis to treatment (Canobbio et al. 1995; Jones et al. 1993; Lopez Hanninen et al. 1996; Mani et al. 1995; Motzer et al. 2002; Palmer et al. 1992). Five studies showed that the time interval between the initial diagnosis of a tumor and the occurrence or start of treatment of the metastatic disease is an independent prognostic factor (Atzpodien et al. 2003; Jones et al. 1993; Motzer et al. 2002; Negrier et al. 2002; Palmer et al. 1992).

Previous treatment characteristics
All studies used in the current review have analyzed the impact of prior nephrectomy to the prognosis of further survival, but most of them did not show that characteristic to be an independent prognostic factor. In only one study by Jones et al., was prior nephrectomy identified as an independent prognostic factor (Jones et al. 1993). The positive impact of nephrectomy for survival in patients with metastatic RCC has been demonstrated in two randomized studies (Flanigan et al. 2001; Mickisch et al. 2001b). Other factors characterizing previous therapy as prior radiotherapy, prior chemo-, immuno- or hormone therapy have been analyzed, but their impact has not been statistically significant (Atzpodien et al. 2003; Canobbio et al. 1995; Jones et al. 1993; Lissoni et al. 1994; Lopez Hanninen et al. 1996; Mani et al. 1995; Motzer et al. 2002; Palmer et al. 1992).

Tumor characteristics
From factors which characterize primary tumor extent or histology such as the initial Robson score (1–2 versus 3–4) (Negrier et al. 2002), primary tumor size (Lopez Hanninen et al. 1996) and sarcomatoid histology (Mani et al. 1995) have

been analyzed. From them only sarcomatoid histology was identified as an independent negative prognostic factor. The study by Mian et al. evaluated prognostic factors for patient survival and the effect of treatment on patient outcome separately in case of sarcomatoid histology with conclusions that patients with sarcomatoid RCC have poor overall survival because of the aggressive biological behavior (Mian et al. 2002).

Characteristics of metastatic process

Five studies have identified the number of metastatic sites as an independent prognostic factor (Atzpodien et al. 2003; Canobbio et al. 1995; Jones et al. 1993; Negrier et al. 2002; Palmer et al. 1992), and they all used in comparison 1 metastatic site versus 2 or more sites, except the study by Atzpodien et al., who analyzed <3 versus ≥3 metastatic sites. Seven studies analyzed the presence of liver, lung, or bone metastases. Two of them revealed the presence of hepatic metastases (Lissoni et al. 1994; Negrier et al. 2002) and four the presence of bone metastases (Atzpodien et al. 2003; Lopez Hanninen et al. 1996; Mani et al. 1995; Negrier et al. 2002) as an independent negative prognostic factor. Only one study has demonstrated significance of only extrapulmonary metastases (Lopez Hanninen et al. 1996).

Laboratory characteristics

A low hemoglobin level below the normal limit or less than 100 mg/L has been identified as an independent prognostic factor in three studies (Lopez Hanninen et al. 1996; Motzer et al. 2002; Negrier et al. 2002). Signs of inflammation characterized by high sedimentation rate and C-reactive protein were identified as independent risk factors in analyses by Negrier et al. (Negrier et al. 2002), C-reactive protein by Atzpodien et al. (Atzpodien et al. 2003) and sedimentation rate over 70 mm/h by Lopez Hanninen et al. (Lopez Hanninen et al. 1996). A high neutrophil count was identified in all three studies, where a respective analysis was performed, as a significant prognostic factor (Atzpodien et al. 2003; Lopez Hanninen et al. 1996; Negrier et al. 2002). Elevated lactate dehydrogenase was also identified as an independent prognostic factor by Motzer et al. (more than 1.5 from upper normal limit) (Motzer et al. 2002), by Lopez Hanninen et al. (more than 280 U) (Lopez Hanninen et al. 1996), and by Atzpodien et al. (< 220 U versus ≥220 U) (Atzpodien et al. 2003). From three studies, only in one was an elevated alkaline phosphatase level identified as an independent factor (Negrier et al. 2002). The study by Motzer et al. identified high corrected serum calcium as an independent factor (Motzer et al. 2002).

5.3. Efficacy and safety of capecitabine and INFa2A combination

Patient characteristics, efficacy results, and toxicity data of the phase II clinical trial with capecitabine and INFa2A are described in Publication III (3. Results). Table 1, Publication III, lists characteristics of the 25 included patients. The overall response rate was 24.0% (95% CI, 9.4–45.1%); from 6 responded patients 5 had partial and 1 complete response. Stable disease status for at least two cycles of therapy was achieved in 9 patients (36.0% with 95% CI 18.0–57.5%). The median survival time was 248 days (95% CI, 173 to 265 days) (Figure 1).

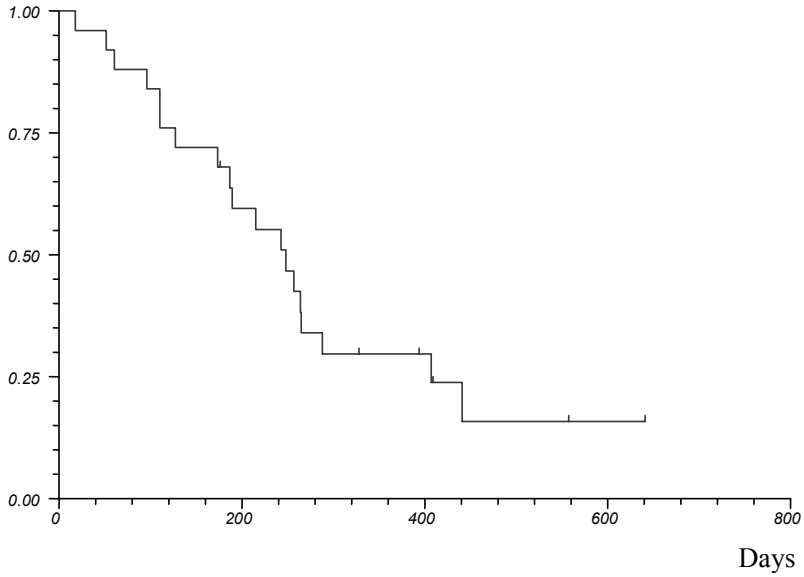

Figure 1. Kaplan-Meier curve of overall survival. Median survival was 248 days.

Table 2, Publication III, characterizes the toxicity of the regimen, NCI-CTC grade 3–4 toxicities occurred in 12 patient and included fatigue (33.3%), nausea, hand-foot syndrome (both 12.5%), anorexia (8.3%), vomiting, anemia, and neutropenia (all 4.2%).

5.4. Results of the thymidine phosphorylase expression analysis

Patients' characteristics

From 25 patients in the phase II trial with INFa2A and capecitabine 16 gave additional informed consent for TP expression analysis. The median age of these patients was 56 years (range, 41 to 67), 12 were males and 4 females, and Eastern Cooperative Oncology Group (ECOG) performance status was 0–2. The median number of three-week cycles was 4.75 (range, 2 to 12). Table 2 lists characteristics of the 16 included patients.

Table 2. Patients' characteristics in TP expression analysis

Characteristics		**No. of patients**	**%**
Total no. of patients		16	
Age, years			
	Mean	56	
	Range	41–67	
Sex			
	Male	12	75.0
	Female	4	25.0
ECOG performance status			
	0	6	37.5
	1	9	56.3
	2	1	6.3
Prior nephrectomy			
	Yes	14	87.5
	No	2	12.5
Evaluable sites			
	Lung	11	68.8
	Liver	3	18.8
	Bone	2	12.5
	Lymph nodes	6	37.5
	Primary tumor	2	12.5
	Local recurrence	1	6.3
	Subcutaneous soft tissue	1	6.3
No. of metastatic sites			
	1	11	68.8
	2	1	6.3
	3	3	18.8
	≥4	1	6.3
No. of treatment cycles			
	Total	76	
	Range	2 to 12	
	Median	4.75	

Overall partial response rate in this group of patients was 31%, stable disease status was additionally achieved in 33 % of patients. Substantial TP expression in tumor tissue was detected in majority of patients, 4 patients had semi-quantitative score 2 or 3 and 12 patients from 4 to 6. All semi-quantitative scores and their relation to treatment effects are characterized in Table 3. No correlation between TP expression and response to treatment with capecitabine and INFa2A was observed in the analyzed group of patients.

Table 3. All semi-quantitative scores and their relation to treatment effects.

		Best response (No. of patients)		
Score	No. of patients	Partial response	Stable disease	Progressive disease
2+	2	1	–	1
3+	2	1	–	1
4+	3	–	1	2
5+	3	–	2	1
6+	6	3	1	2
Total	16	5	4	7

6. DISCUSSION

6.1. Changes in the natural history of renal cell carcinoma

An improvement in 5-year relative survival of renal cancer from 44% in 1978–1980 to 57% in 1990–1994 has been noted in European countries (Damhuis & Kirkels 1998; Sant et al. 2003). Survival rates for Estonian patients have been among the lowest in EUROCARE studies: the 5-year relative survival rate for men and for women was 30% in the period 1985–1989 and 34% for men and 53% for women in 1990–1994 (Damhuis & Kirkels 1998; Sant et al. 2003). Nevertheless, considerable changes have taken place: the 5-year relative survival rate increased in Estonia from about 25% to more than 50% (Aareleid & Brenner 2002). We performed the analysis to compare the stage distribution of RCC and the diagnostic and treatment methods used in Estonia separately in the 1980s and 1990s for explaining the differences in survival.

The most important determinant of prognosis for a patient with RCC is the pathologic stage when tumor is initially diagnosed and treated, other important factors are tumor grade and patient performance status (Lam et al. 2006; Pantuck et al. 2001). It has been proposed that variation in survival rates according to the country or time is probably related to differences in the distribution of tumor stage at diagnosis (Aareleid & Brenner 2002; Damhuis & Kirkels 1998). The increase in the number of the early disease stages may be mainly due to improved diagnostic techniques that are able to detect small tumors. Direct evidence to confirm this theory has been lacking.

The collected data showed that there was a statistically significant difference in stage distribution with an increase for stages I and II and a decrease for stages III and IV in the later period in comparison with the earlier. This difference was significant both for men and women. Our analysis confirmed that survival improved significantly from period 1986–1988 to period 1996–1998, 5-year survival being 23.8% and 47.2% and median survival being 12.0 and 48.0 months, respectively. Survival was higher for both men and women and also in age groups ≥65 and <65 years. Higher survival was also noted for the early disease stages in both periods. Analysis showed no differences in survival between stages I–III in earlier period and between stages I and II in later period. This may be due to lack of statistical power of analysis by stages in the period of 1986–1988, also there can be understaging of disease due to inadequate diagnostics.

Our analysis confirms a shift in the diagnostic methods used for RCC diagnosis. In the period of 1986–1988, the primary method was intravenous urography, followed by renal angiography and abdominal US. In the period of 1996–1998, the dominant primary diagnostic method was already abdominal US. While in the period of 1986–1988 CT was not available, in the period of 1996–1998 it was employed in the majority of patients and yielded primarily

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diagnosis in 10.8% of cases. In later period, angiography and intravenous urography were used only sporadically.

Radical nephrectomy has been shown to result in higher survival rates than simple nephrectomy in the treatment of RCC (Mickisch et al. 2001a; Richie & Kantoff 2003; Stewart & Kleihues 2003). If surgery cannot eradicate all tumor deposits, nephrectomy remains a palliative therapy and should be considered in the context of multimodality therapy (e.g. in conjunction with immunotherapy) (Mickisch et al. 2001a). The data from our study show that in the period of 1996–1998 far more patients were operated for primary tumor removal than ten years earlier. The rise in operation rate was due to the higher operation rate among the stage IV patients. Comparison of the operated and non-operated patients with stage IV disease revealed a significantly higher survival for the operated patients in the period of 1996–1998, but not in the period of 1986–1988. We also detected a change in the operating techniques from simple to radical nephrectomy. This may be also one of the reasons for the higher survival in the period of 1996–1998, although due to different stage distributions, retrospective comparison of survival in the case of different operation methods was not appropriate.

As routine use of immunotherapy for metastatic cases was not available in either study period, this treatment method could not influence the survival differences between the periods. It was not possible to collect reliable data about performance status of patients retrospectively. Also, the tumor grade and histological type data were insufficient for valid comparative analysis.

6.2. Prognostic factors of immunotherapy in metastatic renal cell carcinoma

Determining prognostic factors of survival or of rapid progression under treatment would be of help for selecting patients for immunotherapy to avoid treating patients who will not, in the end, benefit from the treatment. Clinical trials that include survival as an end point must also account for prognostic factors to make sure that treatment groups are comparable, so that the proper interpretation of the trial outcome can be ascertained. So far several studies have analyzed various prognostic factors and have received various results. The choice of analyzed factors has been different in various clinical trials and there is no one summarizing study. As the second part of the current thesis, the role of prognostic and predictive factors of immunotherapy in metastatic RCC is reviewed and analyzed using data from 9 published studies. For this purpose, a comparison between all the analysed prognostic factors and those that have demonstrated an independent prognostic significance in multivariate analyses was performed. Only studies where treatment contained immunotherapy were focused on for this comparison. Since they have been identified in at least 3

independent series, 7 factors appear as the most consistent prognostic factors of survival in patients receiving immunotherapy in metastatic RCC:

- performance status
- time interval from initial diagnosis of tumor to occurrence or start of treatment of metastatic disease
- number of metastatic sites
- presence of bone metastases
- low hemoglobin level
- elevated neutrophil count
- elevated lactate dehydrogenase level.

Less investigated, the laboratory parameters – signs of inflammation (elevated sedimentation rate and C-reactive protein) and corrected serum calcium level, and also sarcomatoid histology – have prognostic importance. More conflicting results have shown the presence of liver and lung metastases and an elevated alkaline phosphatase level. On the other hand, the prognostic factors reported to predict better survival in patients treated with immunotherapy are mostly the same factors that in other series of patients treated with chemotherapy, or even not treated at all, identified patients with longer survival. Therefore, only a controlled prospective study could assess the importance of the selection of a relatively good prognostic factor category of patients in determining the apparent results of the treatment itself. A group of international investigators has been formed to create a comprehensive database of >4,000 patients with metastatic RCC in order to provide and validate a single model that can be used to predict survival. This project is currently under way (Bukowski et al. 2004).

6.3. Combination therapy with capecitabine and INFa2A

During a long time patients with RCC have been treated with IFNa or IL-2 monotherapy, or their combinations outside of clinical trials (Bukowski 2001; Heinzer et al. 2001). Several studies have shown a survival benefit for patients treated with IFNa (Pyrhonen et al. 1999; Medical Research Council Renal Cancer Collaborators 1999). As therapeutic effects of immunotherapy are limited, investigation of new agents with potential treatment efficacy has had vital importance.

Capecitabine is an orally administered fluoropyrimidine carbamate capable of exploiting the high concentrations of TP in tumor tissue to achieve activation preferentially at the tumor site (Ishikawa et al. 1998; Mackean et al. 1998; Miwa et al. 1998; Schuller et al. 2000). The rationale of investigating capecitabine use in RCC lies in the fact that tumor types known to have a high level of TP activity, such as renal cancer, are possible targets for capecitabine therapy (Twelves 2001). Capecitabine also has potential as a combination

partner for IFNa immunotherapy with different mechanisms of action, possible synergism, and little overlapping of key toxicities. The combination of capecitabine with subcutaneous recombinant human IFNa2A, recombinant human IL-2, and oral 13-cis-retinoic acid has shown promising efficacy in the first-line treatment of metastatic RCC (Oevermann et al. 2000), as well as monotherapy after immunotherapy failure (Wenzel et al. 2002). However, it is not clear to what extent the treatment results are interrelated to the capecitabine effect, and if the 13-cis-retinoic acid is necessary in the regimen.

We performed the prospective single institution phase II study to evaluate the efficacy and safety of capecitabine and IFNa2A combination as the first line treatment in patients with metastatic RCC. A phase I study by Chang et al. of capecitabine and IFNa2A has demonstrated moderate toxicity with recommended dosage levels for phase II trials 3.0 million U/m² subcutaneously three times weekly for IFNa2A and 1,000 mg/m² twice daily for 2 weeks for oral capecitabine (Chang et al. 2001). In our study the capecitabine dose and treatment schedule was chosen to be the same as that which is recommended for breast and colorectal cancer monotherapy (1,250 mg/m² twice daily for 14 days followed by a 7-day rest period), and was combined with IFNa2A administered subcutaneously at 6 million U three times weekly. Our choice of capecitabine dosage was based on a good toxicity profile in a study by Wenzel et al. (Wenzel et al. 2002) and under the circumstance that IFNa2A dose was chosen relatively low. We observed an overall response rate of 24.0% (95% CI, 9.4–45.1%); there were 5 partial responses and 1 complete response. The median survival time was 248 days (95% CI, 173 to 265 days). Phase III randomized trials of metastatic RCC, using in their arms IFNa monotherapy or IFNa combinations with nephrectomy, have demonstrated objective response rates from 3.3% to 19% and overall median survival from 7 to 17 months (De Mulder et al. 1995; Flanigan et al. 2001; Fossa et al. 1992; Medical Research Council Renal Cancer Collaborators 1999; Mickisch et al. 2001b; Motzer et al. 2000; Negrier et al. 1998). The response rate and median survival detected in our study remain within efficacy results of these studies. Response proportions in patients with metastatic RCC to immunotherapy programs vary considerably among clinical trials (Bukowski 2000; Bukowski 2001; Coppin et al. 2000; Fossa 2000), implying that patient selection is an important factor in achieving a favorable treatment outcome. Therefore, only a randomized trial can give the final answer about concrete regimen efficacy in comparison with standard therapy. The toxicity of the regimen was not low, but was still relatively tolerable. Capecitabine dose reduction was necessary in 12 (48%) and IFNa2A dose reduction in 5 patients (20%). Because of the relatively high rate of necessary capecitabine dose reductions, a more justified dose of capecitabine for further studies may be 1,000 mg/m² twice daily for 14 days followed by 7 days of rest. A commonly observed and characteristic toxicity for capecitabine is hand-foot syndrome. We observed grade 1–2 hand-foot syndrome in 5 (20.8%) and grade

3 in 3 (12.5%) patients, which is not more than described in phase III colorectal and breast cancer studies (Hoff et al. 2001; O'Shaughnessy et al. 2002; Van Cutsem et al. 2001). All cases of hand-foot syndrome were manageable with therapy interruption or dose reduction. We concluded that capecitabine and IFNa2A combination has clinical activity and a relatively acceptable toxicity profile in patients with metastatic RCC. The importance of adding capecitabine to IFNa2A needs to be further evaluated in a randomized trial.

6.4. Thymidine phosphorylase expression in renal cell carcinoma tissue

TP is the enzyme that metabolizes 5'-deoxy-5-fluorouridine, an intermediate metabolite of capecitabine, to the active drug 5-fluorouracil. Several studies have demonstrated that the enzymatic activity of TP is higher in RCC tissue than in normal kidney tissue (Imazano et al. 1997; Mizutani et al. 2003; Morita et al. 2003). In vitro studies have also demonstrated that there is a significant positive correlation between the TP activity and sensitivity to 5-fluorouracil in RCC, suggesting that TP may be useful as a predictive factor in combination therapy with INFa and 5-fluorouracil (Hirano et al. 2003; Ikemoto et al. 2002). Another in vitro study by Morita et al. provided direct evidence that TP plays a role in mediating the sensitivity of RCC to capecitabine (Morita et al. 2001). All these results implied that TP expression measurement in RCC could identify subgroups of metastatic RCC which may respond to INFa and capecitabine combination therapy. As capecitabine is capable of exploiting the high concentrations of TP in tumor tissue to achieve activation preferentially at the tumor site, the purpose of this part of study was to implement TP expression analysis in RCC tissue with immunohistochemistry assays with monoclonal anti-TP antibody, to evaluate the level of TP expression and possible correlation between TP values and treatment efficacy of capecitabine and INFa2A combination. Substantial TP expression in tumor tissue was detected in the majority of patients with metastatic RCC. Responses to the treatment occurred both in patients with low TP expression level as well as in patients with a high level. No correlation between TP expression and response to treatment with capecitabine and INFa was possible to observe in the analyzed group of patients. Partial response to treatment in patients with low level of TP could be due to independent treatment effect of INFa, also our patients' group was very small and a larger study is needed. TP expression evaluation by immunohistochemistry assays is a diagnostic method, which is easily applicable in everyday practice.

7. CONCLUSIONS

1. The increase in the survival of Estonian RCC patients from the period 1986–1988 to 1996–1998 has been due to the larger number of cases with early disease stage, which is associated with the application of US and CT. Another factor for better survival was the higher operation rate among patients with stage IV disease. There was also a change in operation techniques towards routine use of radical nephrectomy: the large proportion of simple nephrectomies in the earlier period could be one of the reasons for the lower survival in the past.

2. The most consistent prognostic factors of survival in patients receiving immunotherapy in metastatic RCC are:
   - performance status
   - time interval from initial diagnosis of tumor to occurrence or start of treatment of metastatic disease
   - number of metastatic sites
   - presence of bone metastases
   - low hemoglobin level
   - elevated neutrophil count
   - elevated lactate dehydrogenase level.

3. Capecitabine and IFNa2A combination has clinical activity and a relatively acceptable toxicity profile in the treatment of patients with metastatic RCC. Because of the relatively high rate of necessary capecitabine dose reductions, a more justified dose of capecitabine for further studies is 1,000 mg/m² twice daily for 14 days followed by 7 days of rest.

4. Substantial TP expression in tumor tissue was detected in the majority of patients with metastatic RCC. No correlation between TP expression and response to treatment with capecitabine and IFNa2A was possible to detect in the analyzed group of patients.

8. REFERENCES

Aareleid T, Brenner H. Trends in cancer patient survival in Estonia before and after the transition from a Soviet republic to an open-market economy. Int J Cancer 2002; 102 (1):45–50.

Aareleid T, Mägi M, editors. Cancer Incidence in Estonia 1996 Tallinn: Estonian Cancer Registry; 1999.

Aareleid T, Mägi M, editors. Cancer Incidence in Estonia 1997 Tallinn: Estonian Cancer Registry; 2000.

Aareleid T, Mägi M, editors. Cancer Incidence in Estonia 1998 Tallinn: Estonian Cancer Registry; 2001.

Aareleid T, Rahu M. Cancer survival in Estonia from 1978 to 1987. Cancer 1991; 68 (9):2088–92.

Aass N, De Mulder PH, Mickisch GH et al. 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 2005; 23 (18):4172–8.

Amato RJ. Chemotherapy for renal cell carcinoma. Semin Oncol 2000; 27 (2):177–86.

Atkins MB, Hidalgo M, Stadler WM et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 2004; 22 (5):909–18.

Atzpodien J, Kirchner H, Jonas U et al. Interleukin-2- and interferon alfa-2a-based immunochemotherapy in advanced renal cell carcinoma: a Prospectively Randomized Trial of the German Cooperative Renal Carcinoma Chemoimmunotherapy Group (DGCIN). J Clin Oncol 2004; 22 (7):1188–94.

Atzpodien J, Royston P, Wandert T et al. Metastatic renal carcinoma comprehensive prognostic system. Br J Cancer 2003; 88 (3):348–53.

Bukowski RM. Cytokine combinations: therapeutic use in patients with advanced renal cell carcinoma. Semin Oncol 2000; 27 (2):204–12.

Bukowski RM. Cytokine therapy for metastatic renal cell carcinoma. Semin Urol Oncol 2001; 19 (2):148–54.

Bukowski RM, Negrier S, Elson P. Prognostic factors in patients with advanced renal cell carcinoma: development of an international kidney cancer working group. Clin Cancer Res 2004; 10 (18 Pt 2):6310S–4S.

Canobbio L, Rubagotti A, Miglietta L et al. Prognostic factors for survival in patients with advanced renal cell carcinoma treated with interleukin-2 and interferon-alpha. J Cancer Res Clin Oncol 1995; 121 (12):753–6.

Chang DZ, Olencki T, Budd GT et al. Phase I trial of capecitabine in combination with interferon alpha in patients with metastatic renal cancer: toxicity and pharmacokinetics. Cancer Chemother Pharmacol 2001; 48 (6):493–8.

Chikkala NF, Lewis I, Ulchaker J et al. Interactive effects of alpha-interferon A/D and interleukin 2 on murine lymphokine-activated killer activity: analysis at the effector and precursor level. Cancer Res 1990; 50 (4):1176–82.

Coppin C, Porzsolt F, Awa A et al. Immunotherapy for advanced renal cell cancer. Cochrane Database Syst Rev 2005; (1):CD001425.

Coppin C, Porzsolt F, Kumpf J et al. Immunotherapy for advanced renal cell cancer. Cochrane Database Syst Rev 2000; (3):CD001425.

Damhuis RA, Kirkels WJ. Improvement in survival of patients with cancer of the kidney in Europe. Eur J Cancer 1998; 34 (14):2232–5.

De Mulder PH, Oosterhof G, Bouffioux C et al. EORTC (30885) randomised phase III study with recombinant interferon alpha and recombinant interferon alpha and gamma in patients with advanced renal cell carcinoma. The EORTC Genitourinary Group. Br J Cancer 1995; 71 (2):371–5.

Eble JN, Sauter G, Epstein JI, Sesterhenn IA, eds. In: Pathology and genetics of tumours of the urinary system and male genital organs. World Health Organization Classification of Tumours. Lyons: IARC Press, 2004, p. 7.

Eisen T, Staehler M, Szczylik C, et al. Randomized phase III trial of sorafenib in advanced renal cell carcinoma (RCC): Impact of crossover on survival. Proc Am Soc Clin Oncol 2006 2006:Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 4524.

Escudier B, Szczylik C, Eisen T et al. Randomized phase III trial of the multikinase inhibitor sorafenib (BAY 43–9006) in patients with advanced renal cell carcinoma (RCC). Eur J Cancer 2005; 3:226 (Abstr 794).

Estonian Cancer Registry. Data in File, 2006.

Flanigan RC, Salmon SE, Blumenstein BA et al. Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. N Engl J Med 2001; 345

Fossa SD. Interferon in metastatic renal cell carcinoma. Semin Oncol 2000; 27 (2):187–93.

Fossa SD, Martinelli G, Otto U et al. Recombinant interferon alfa-2a with or without vinblastine in metastatic renal cell carcinoma: results of a European multi-center phase III study. Ann Oncol 1992; 3 (4):301–5.

Fyfe G, Fisher RI, Rosenberg SA et al. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995; 13 (3):688–96.

Gleave ME, Elhilali M, Fradet Y et al. Interferon gamma-1b compared with placebo in metastatic renal-cell carcinoma. Canadian Urologic Oncology Group. N Engl J Med 1998; 338 (18):1265–71.

Haarstad H, Jacobsen AB, Schjolseth SA et al. Interferon-alpha, 5-FU and prednisone in metastatic renal cell carcinoma: a phase II study. Ann Oncol 1994; 5 (3):245–8.

Hartmann JT, Bokemeyer C. Chemotherapy for renal cell carcinoma. Anticancer Res 1999; 19 (2C):1541–3.

Heinzer H, Huland E, Huland H. Systemic chemotherapy and chemoimmunotherapy for metastatic renal cell cancer. World J Urol 2001; 19 (2):111–9.

Hirano Y, Takayama T, Kageyama S et al. Thymidine phosphorylase and di-hydropyrimidine dehydrogenase in renal cell carcinoma: relationship between histological parameters and chemosensitivity to 5-fluorouracil. Eur Urol 2003; 43 (1):45–51; discussion –2.

Hoff PM, Ansari R, Batist G et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J Clin Oncol 2001; 19 (8):2282–92.

Hudes G, Carducci M, Tomczak P et al. A phase III, randomized, 3-arm study of temsirolimus (TEMSR) or interferon-alpha (IFN) or the combination of TEMSR + IFN in the treatment of first-line, poor-risk patients with advanced renal cell carcinoma (advRCC). J Clin Oncol 24:930s, 2006 (suppl; abstr LBA4).

Ikemoto S, Sugimura K, Yoshida N et al. Comparative antitumor activity of 5-fluorouracil and 5'-deoxy-5-fluorouridine in combination with interferon gamma in renal cell carcinoma cell lines. Anticancer Res 2002; 22 (6C):4023–7.

Imazano Y, Takebayashi Y, Nishiyama K et al. Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma. J Clin Oncol 1997; 15 (7):2570–8.

Ishikawa T, Sekiguchi F, Fukase Y et al. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 1998; 58 (4):685–90.

Jones M, Philip T, Palmer P et al. The impact of interleukin-2 on survival in renal cancer: a multivariate analysis. Cancer Biother 1993; 8 (4):275–88.

Kriegmair M, Oberneder R, Hofstetter A. Interferon alfa and vinblastine versus medroxyprogesterone acetate in the treatment of metastatic renal cell carcinoma. Urology 1995; 45 (5):758–62.

Lam JS, Breda A, Belldegrun AS et al. Evolving principles of surgical management and prognostic factors for outcome in renal cell carcinoma. J Clin Oncol 2006; 24 (35):5565–75.

Lam JS, Shvarts O, Leppert JT et al. Renal cell carcinoma 2005: new frontiers in staging, prognostication and targeted molecular therapy. J Urol 2005; 173 (6):1853–62.

Lissoni P, Barni S, Ardizzoia A et al. Prognostic factors of the clinical response to subcutaneous immunotherapy with interleukin-2 alone in patients with metastatic renal cell carcinoma. Oncology 1994; 51 (1):59–62.

Lopez Hanninen E, Kirchner H, Atzpodien J. Interleukin-2 based home therapy of metastatic renal cell carcinoma: risks and benefits in 215 consecutive single institution patients. J Urol 1996; 155 (1):19–25.

Mackean M, Planting A, Twelves C et al. Phase I and pharmacologic study of intermittent twice-daily oral therapy with capecitabine in patients with advanced and/or metastatic cancer. J Clin Oncol 1998; 16 (9):2977–85.

Mani S, Todd MB, Katz K et al. Prognostic factors for survival in patients with metastatic renal cancer treated with biological response modifiers. J Urol 1995; 154 (1):35–40.

Margolin KA. Interleukin-2 in the treatment of renal cancer. Semin Oncol 2000; 27 (2):194–203. Medical Research Council Renal Cancer Collaborators. Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Lancet 1999; 353 (9146):14–7.

Mian BM, Bhadkamkar N, Slaton JW et al. Prognostic factors and survival of patients with sarcomatoid renal cell carcinoma. J Urol 2002; 167 (1):65–70.

Mickisch GH, Carballido J, Hellsten S et al. Guidelines on renal cell cancer. Eur Urol 2001a; 40 (3):252–5.

Mickisch GH, Garin A, van Poppel H et al. Radical nephrectomy plus interferon-alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. Lancet 2001b; 358 (9286):966–70.

Mizutani Y, Wada H, Yoshida O et al. The significance of thymidine phosphorylase/ platelet-derived endothelial cell growth factor activity in renal cell carcinoma. Cancer 2003; 98 (4):730–6.

Miwa M, Ura M, Nishida M et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 1998; 34 (8):1274–81.

Morita T, Matsuzaki A, Tokue A. Enhancement of sensitivity to capecitabine in human renal carcinoma cells transfected with thymidine phosphorylase cDNA. Int J Cancer 2001; 92 (3):451–6.

Morita T, Matsuzaki A, Tokue A. Quantitative analysis of thymidine phosphorylase and dihydropyrimidine dehydrogenase in renal cell carcinoma. Oncology 2003; 65 (2):125–31.

Morita T, Tokue A. Biomodulation of 5-fluorouracil by interferon-alpha in human renal carcinoma cells: relationship to the expression of thymidine phosphorylase. Cancer Chemother Pharmacol 1999; 44 (2):91–6.

Motzer RJ. Renal cell carcinoma: progress against an elusive tumor. Semin Oncol 2000; 27 (2):113–4.

Motzer RJ, Bacik J, Murphy BA et al. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol 2002; 20 (1):289–96.

Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N Engl J Med 1996; 335 (12):865–75.

Motzer RJ, Murphy BA, Bacik J et al. Phase III trial of interferon alfa-2a with or without 13-cis-retinoic acid for patients with advanced renal cell carcinoma. J Clin Oncol 2000; 18 (16):2972–80.

Motzer RJ, Tomczak P, Michaelson MD et al. Phase III randomized trial of sunitinib malate (SU11248) versus interferon-alfa (IFN-α) as first-line systemic therapy for patients with metastatic renal cell carcinoma (mRCC). ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: LBA3 2006.

Negrier S, Escudier B, Gomez F et al. Prognostic factors of survival and rapid progression in 782 patients with metastatic renal carcinomas treated by cytokines: a report from the Groupe Francais d'Immunotherapie. Ann Oncol 2002; 13 (9):1460–8.

Negrier S, Escudier B, Lasset C et al. Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d'Immunotherapie. N Engl J Med 1998; 338 (18):1272–8.

Negrier S, Maral J, Drevon M et al. Long-term follow-up of patients with metastatic renal cell carcinoma treated with intravenous recombinant interleukin-2 in Europe. Cancer J Sci Am 2000; 6 (Suppl 1):S93–8.

Negrier S, Perol D, Ravaud A et al. Do cytokines improve survival in patients with metastatic renal cell cancer of intermediate prognosis? Results of the prospective randomized PERCY Quattro trial. Proc Am Soc Clin Oncol 2005; LBA4511 Abstr). 2005.

Neidhart JA, Anderson SA, Harris JE et al. Vinblastine fails to improve response of renal cancer to interferon alfa-n1: high response rate in patients with pulmonary metastases. J Clin Oncol 1991; 9 (5):832–6.

O'Shaughnessy J, Miles D, Vukelja S et al. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol 2002; 20 (12):2812–23.

Oevermann K, Buer J, Hoffmann R et al. Capecitabine in the treatment of metastatic renal cell carcinoma. Br J Cancer 2000; 83 (5):583–7.

Palmer PA, Vinke J, Philip T et al. Prognostic factors for survival in patients with advanced renal cell carcinoma treated with recombinant interleukin-2. Ann Oncol 1992; 3 (6):475–80.

Pantuck AJ, Zisman A, Belldegrun AS. The changing natural history of renal cell carcinoma. J Urol 2001; 166 (5):1611–23.

Parkin DM, Bray F, Ferlay J et al. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001; 94 (2):153–6.

Parkin DM, Pisani P, Ferlay J. Global cancer statistics. CA Cancer J Clin 1999; 49 (1):33–64, 1.

Pittman K, Selby P. The management of renal cell carcinoma. Crit Rev Oncol Hematol 1994; 16 (3):181–200.

Pyrhonen S, Salminen E, Ruutu M et al. Prospective randomized trial of interferon alfa-2a plus vinblastine versus vinblastine alone in patients with advanced renal cell cancer. J Clin Oncol 1999; 17 (9):2859–67.

Richie JP, Kantoff PW. Renal Cell Carcinoma. In: WD Kufe; RE Pollock; RR Weichselbaum et al., editors, translator and editor Cancer Medicine. 6th edn. Vol. 2: BC Decker Inc; 2003; p. 1675–82.

Robson CJ, Churchill BM, Anderson W. The results of radical nephrectomy for renal cell carcinoma. Trans Am Assoc Genitourin Surg 1968; 60:122–9.

Sant M, Aareleid T, Berrino F et al. EUROCARE-3: survival of cancer patients diagnosed 1990–94--results and commentary. Ann Oncol 2003; 14 Suppl 5:v61–118.

Schomburg A, Kirchner H, Fenner M et al. Lack of therapeutic efficacy of tamoxifen in advanced renal cell carcinoma. Eur J Cancer 1993; 29A (5):737–40.

Schuller J, Cassidy J, Dumont E et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. Cancer Chemother Pharmacol 2000; 45 (4):291–7.

Stahl M, Wilke H, Schmoll HJ et al. A phase II study of high dose tamoxifen in progressive, metastatic renal cell carcinoma. Ann Oncol 1992; 3 (2):167–8.

Steineck G, Strander H, Carbin BE et al. Recombinant leukocyte interferon alpha-2a and medroxyprogesterone in advanced renal cell carcinoma. A randomized trial. Acta Oncol 1990; 29 (2):155–62.

Stewart BW, Kleihues P, editors. World Cancer Report Lyon: IARC Press; 2003.

Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000; 92 (3):205–16.

Twelves C. Vision of the future: capecitabine. Oncologist 2001; 6 Suppl 4:35–9.

Van Cutsem E, Twelves C, Cassidy J et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. J Clin Oncol 2001; 19 (21):4097–106.
Wenzel C, Locker GJ, Schmidinger M et al. Capecitabine in the treatment of metastatic renal cell carcinoma failing immunotherapy. Am J Kidney Dis 2002; 39 (1):48–54.
Vogelzang NJ, Stadler WM. Kidney cancer. Lancet 1998; 352 (9141):1691–6.
Yagoda A, Abi-Rached B, Petrylak D. Chemotherapy for advanced renal-cell carcinoma: 1983–1993. Semin Oncol 1995; 22 (1):42–60.
Yang JC, Childs R. Immunotherapy for renal cell cancer. J Clin Oncol 2006; 24 (35):5576–83.
Yang JC, Haworth L, Sherry RM et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003; 349 (5):427–34.

SUMMARY IN ESTONIAN

Neerurakk-kartsinoom: Haiguse kulu muutused ja metastaatilise haiguse ravi

Sissejuhatus

Maailmas haigestub neeruvähki igal aastal 190 000 ja sureb neeruvähi tõttu 91 000 inimest, neeruvähk moodustab 1,9% kõigist uutest vähijuhtudest ja 1,5% vähist põhjustatud surmajuhtudest. Sagedasimaks neeru pahaloomuliseks kasvajaks on neerurakk-kartsinoom (NRK), mis moodustab 85% neeru pahaloomulistest kasvajatest.

Viimastel aastakümnetel on neeru pahaloomulistesse kasvajatesse haigestumus püsivalt tõusnud, nii tõusis Eestis standarditud haigestumus 6,0-lt kuni 17,2 haigusjuhuni 100 000 eleniku kohta meestel ja 2,2-lt kuni 7,2 juhuni naistel. Samas on 5-aasta elulemus kasvanud 25%-lt üle 50%-ni. Kuigi sarnane muutus on täheldatav ka teistes riikides, on Eesti näitajate muutuse ulatus tähelepanuväärne.

Piirdunud haiguse korral on NRK valikraviks radikaalne nefrektoomia, väiksemate kasvajate puhul ka neeru resektsioon. Kaugmetastaaside korral kasutatakse algkolde eemaldamise ja süsteemse medikamentoosse ravi kombinatsiooni.

NRK on keemiaravile resistentne kasvaja, näidates enamike tsütostaatikumide suhtes marginaalset ravivastust. Enim kasutatud ja parimaid tulemusi andnud süsteemne ravi metastaseerunud NRK korral on olnud immuunravi, patsiente ravitakse väljaspool kliinilisi uuringuid interferoon-alfa (INFa) või interleukiin-2 (IL-2) monoteraapia või kombinatsioonidega. Kliinilised uuringud on näidanud, et INFa kasutamine parandab elulemust, näidates paremaid ravitulemusi võrreldes hormoonravi või vinblastiini monoteraapiaga. Keskmine ravivastus INFa ravile on 15%. IL-2 on näidanud täielikke ravivastuseid 5–7%-l ja koguravivastuseid ligikaudu 15%-l juhtudest, kuid puuduvad elulemuse paranemist näitavad randomiseeritud uuringud. Uute ravivõimalustena metastaatilise NRK ravis on lisandunud türosiinkinaasi inhibiitorid sunitinib ja sorafenib, uuringutes on lubavaid tulemusi andnud CCI-779 ja bevacizumab. Seoses asjaoluga, et ravitulemused on siiski piiratud, soovitatakse patsientide ravi metastaseerunud NRK korral viia läbi kliiniliste uuringute raames, et leida uusi ja paremaid ravivõimalusi.

Metastaatilise NRK elulemuse ja ravi prognostilisi faktoreid tuleb kasutada nii kliiniliste raviotsuste tegemisel kui ka kliiniliste uuringute disainimisel ja interpreteerimisel. Süsteemse ravi eesmärk NRK korral on pikendada elulemust ilma elukvaliteeti negatiivselt mõjutamata. NRK immuunravi kliinilised uuringud näitavad, et ravi positiivne toime esineb väikesel osal patsientidest, samas

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aga esinevad enamikul patsientidest immuunravist tingitud kõrvaltoimed. Seetõttu on oluline leida faktorid, mis aitaks ette ennustada ravi positiivset toimet, et vältida kõrvaltoimeid tekitavat ravi neil patsientidel, kellel positiivse raviefekti tõenäosus on väga väike.

Käesolev doktoridissertatsioon hõlmab NRK ravi erinevaid aspekte: teostatud on Eesti erinevate ajaperioodide neeruvähi elulemuse, diagnostika ja ravimeetodite võrdlev analüüs selgitamaks muutusi neeruvähi ravi tulemustes; analüüsitud on erinevate immuunravi uuringute andmete põhjal erinevate kliiniliste ja laboratoorsete näitajate väärtust immuunravi prognostiliste faktoritena; uute ravivõimaluste arendamiseks on läbi viidud II faasi kliiniline uuring kapetsitabiini ja INFa2A kombinatsiooniga koos tümidiinfosforülaasi ekspressiooni analüüsiga uuringusse kaasatud patsientide kasvajakoes.

Uurimistöö eesmärgid

1. Selgitada NRK haigete elulemuse erinevuste põhjused Eestis 1980-tel ja 1990-tel aastatel võrreldes NRK jaotumust staadiumite kaupa ja kasutatud diagnostika ning ravimeetodeid (Publikatsioon I).
2. Leida kõige olulisemad elulemuse prognostilised faktorid metastaatilise NRK immuunravi korral (Publikatsioon II).
3. Hinnata kapetsitabiini ja INFa2A kombinatsioonravi efektiivsust ja ohutust metastaatilise NRK esmavaliku ravina (Publikatsioon III).
4. Hinnata tümidiinfosforülaasi ekspressioonitaset NRK koes ja võimalikku seost selle ja kapetsitabiini ja INFa2A kombinatsioonravi efektiivsuse vahel (Publikatsioon IV).

Patsiendid ja uurimismeetodid

Käesolev väitekiri tuleneb kolmele uuringule ja ühele kirjanduse analüüsile:

1. **Muutused neeruvähi haigestumus- ja elulemusnäitajates, diagnostikas ja ravis**

Läbi on viidud retrospektiivne analüüs kolme Eesti haigla haiguslugude põhjal võrdlemaks NRK patsientide jaotumust haiguse levikuulatuse, kasutatud diagnostika ja ravimeetodite järgi perioodidel 1986–1988 (122 patsienti) ja 1996–1998 (305 patsienti).

2. **Metastaatilise neeruvähi immuunravi prognostilised faktorid**

Teostatud on üheksa publitseeritud immuunravi (IL-2 ja INFa monoteraapiad ja omavahelised kombinatsioonid või kombinatsioonid keemiaraviga) kasutanud seeria põhjal võrdlus kõigi uuritud prognostiliste faktorite ja nende faktorite

vahel, mis on näidanud iseseisvat prognostilist väärtust mitmikfaktoranalüüside põhjal.

3. **II faasi kliiniline uuring kapetsitabiini ja INFa2A kombinatsiooniga**

Kapetsitabiin on suukaudselt manustatav tsütostaatikum, mis muutub aktiivseks ühendiks, 5-fluorouratsiiliks, kudedes ensüümi tümidiinfosforülaasi toimel. Sealjuures on tümidiinfosforülaasi kontsentratsioon kõrge just kasvajakoes, mis võimaldab seal ka tsütostaatikumi suuremat toimet. Tümidiinfosforülaasi kõrget kontsentratsiooni on *ex vivo* uuringutes näidatud ka neerurakulise kartsinoomi koes.

Uuringusse kaasati patsiendi kirjaliku informeeritud nõusoleku järgselt metastaseerunud või lokaalselt levinud mitteresektaabelse NRK patsiendid, kelle näitajad vastasid uuringuprotokollis toodud inklusiooni- ja eksklusiooni-kriteeriumitele. Ravikuur oli kolm nädalat pikk, kapetsitabiini manustati 1250 mg/m² suukaudselt kaks korda päevas 14 päeva, millele järgnes 7-päevane paus, INFa2A manustati nahaalusi 6 miljonit ühikut kolm korda nädalas pidevalt. Ravi jätkati kuni 12 ravikuuri või kuni haiguse progressiooni või mitteaktsepteeritava toksilisuse tekkeni või kui patsient soovis ravi katkestada. Enne ravi alustamist viidi läbi patsiendi füüsikaline uuring, kliinilised ja biokeemilised vereanalüüsid, EKG ja algkolde või metastaaside ulatust hindavad uuringud. Raviefekti hinnati iga kahe ravikuuri järel RECIST kriteeriumite alusel, toksilisust pidevalt *National Cancer Institute* toksilisuse kriteeriumite versioon 2 alusel. Toksilisuse korral modifitseeriti vajadusel ravimite doose. Uuringuprotokoll kiideti heaks Tartu Ülikooli Inimuuringute Eetikakomitee poolt.

Oktoobrist 2001 kuni veebruarini 2003 sisestati uuringusse 25 patsienti keskmise vanusega 57,2 (41 kuni 73) aastat, 18 patsienti olid mehed ja 7 naised. 3-nädalaste ravikuuride keskmine arv oli 5,2 (1 kuni 12).

4. **Tümidiinfosforülaasi ekspressiooni analüüsi rakendamine metastaatilise NRK patsientide kasvajakoes**

Käesoleva uuringuosa eesmärgiks oli hinnata seost tümidiinfosforülaasi ekspressioonitaseme ja raviefekti vahel patsientidel, kellel oli diagnoositud NRK ning kes said kombinatsioonravi kapetsitabiini ja INFa-ga. Tümidiinfosforülaasi ekspressioon määrati täiendava informeeritud nõusoleku andnud 16 patsiendi kasvajakoes immuunohistokeemilise analüüsiga, kasutades poolkvantitatiivset kasvajarakkude skoori süsteemi, kus skoorid arvutati värvunud rakkude protsendi ja värvumusintensiivsuse skooride summana 0-st 7-ni.

Uurimistööst tulenevad järeldused

1. Eesti NRK patsientide elulemuse paranemine perioodis 1996–1998 võrreldes perioodiga 1986–1988 on olnud tingitud varasemas staadiumis olevate haigusjuhtude suuremast osakaalust, mis on seostatav ultraheli ja kompuutertomograafia rakendamisega. Teiseks elulemust parandavaks faktoriks oli opereeritud patsientide suurem osakaal IV staadiumiga haigete osas. Võimaliku faktorina võib välja tuua muutuse operatsioonitehnikas lihtsalt nefrektoomialt radikaalse nefrektoomia valdavale kasutamisele.

2. Kõige püsivamad prognostilist väärtust omavat faktorid metastaatilise NRK immuunravi korral on:

- üldstaatus
- ajaline intervall algsest kasvaja diagnoosist kuni metastaatilise haiguse diagnoosimise või ravini
- metastaatiliste paikmete arv
- luumetastaaside esinemine
- madal hemoglobiini tase
- tõusnud neutrofiilide arv
- tõusnud laktaatdehüdrogenaasi tase.

Vähem uuritud, kuid samuti prognostilist tähtsust omavad kaks laboratoorset parameetrit – põletikunäitajad (kiirenenud settereaktsioon ja C-reaktiivne valk) ja korrigeeritud kaltsiumitase, samuti ka kasvaja sarkomatoidne histoloogiline vorm.

3. Kapetsitabiini ja INFa2A kombinatsioon omab kliinilist aktiivsust ja suhteliselt aktsepteeritavat toksilisuse profiili metastaatilise NRK patsientidel. Kapetsitabiini doosi sagedase redutseerimisvajaduse tõttu on soovitatav doos edasisteks uuringuteks 1000 mg/m² kaks korda päevas 14 päeva vältel järgneva 7 päevase pausiga.

4. Oluline tümidiinfosforülaasi ekspressioon esines enamiku NRK patsientide kasvajakoes. Seost tümidiinfosforülaasi ekspressioonitaseme ja kapetsitabiini ning INFa2A ravivastuse vahel ei olnud uuritud haigete grupil võimalik leida.

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PUBLICATIONS

I

Padrik P, Kirsimagi U, Everaus H. Changes in the natural history of renal cancer: comparison of Estonian data from the periods 1986–1988 and 1996–1998. Int Urol Nephrol 2007; 39 (1):35–41. Epub 2006 Oct 25.

II

Padrik P. Prognostic factors of immunotherapy
in metastatic renal cell carcinoma.
Med Oncol 2003; 20 (4):325–34.

III

Padrik P, Leppik K, Arak A. Combination therapy with capecitabine and interferon alfa-2A in patients with advanced renal cell carcinoma: a phase II study. Urol Oncol 2004; 22 (5):387–92.

IV

Padrik P, Saar H, Leppik K. Application of analysis of thymidine phosphorylase expression in metastatic renal carcinoma tissue. Estonian Physician 2006; 85 (4):297–300 (in Estonian, summary in English).

CURRICULUM VITAE

PEETER PADRIK, MD

Date of birth: 02.02. 1966
Place of birth: Tartu, Estonia
Citizenship: Estonian
Address, phone, e-mail: Vallikraavi 7, Tartu 51003, Tel. 3727319800, peeter.padrik@kliinikum.ee
Place of work and professional position: Director of the Clinic, medical oncologist, Clinic of Hematology and Oncology, Tartu University Hospital

Education

Hugo Treffner Gymnasium, 1984
University of Tartu, Faculty of Medicine, 1990
Internship at the Tartu University Hospital, 1990–1991

Professional career

Tartu University Hospital, Clinic of Hematology and Oncology, Director, since 2000
Tartu University Hospital, Hospital of Oncology, Head Physician, 1997–1999
Tartu University Hospital, Hospital of Oncology, Deputy Head Physician, 1996–1997
Tartu University Hospital, Hospital of Oncology, oncologist, 1993–1996
Estonian Cancer Centre, Tallinn, oncologist, 1992–1993
Estonian Institute of Experimental and Clinical Medicine, junior scientific worker, 1991–1992

Postgraduate professional training

European School of Oncology advanced courses:
Breast Cancer Surgery, 2000; Molecular Biology for Clinicians, 1996; Colorectal Carcinoma,1995; Plastic and Reconstructive Surgery in Oncology, 1994; Cancer of the Pancreas and Liver, 1994; Breast Cancer, 1993
EORTC Residential Course “Cancer Clinical Trials”, 1996
Training at the Clinicum of the University of Göttingen, 1996; at the Hospital of Surgery, University of Helsinki, 1995; at the Odense University Hospital, 1992
Danish Institute of Health Care course “Health Care Management”, 1999
International European and American cancer conferences and courses, 1995–2007

Scientific work

Main scientific research has been on the field of renal, breast and colorectal cancer
Principal investigator in 16 clinical trials
4 papers in international journals, 3 in the "Estonian Physician", 8 abstracts in international conferences

Scientific societies memberships

Estonian Society of Oncologists, since 1992, Member of the Board
American Society of Clinical Oncology, Member since 2000
European Society of Medical Oncology, Member since 2002
Estonian Society of Medical Oncology, since 2006, Chairman of the Board

CURRICULUM VITAE

Dr. PEETER PADRIK

Sünniaeg ja koht: 02.02.1966, Tartu
Kodakondsus: eesti
Aadress, telefon, *e-mail*: Vallikraavi 7, Tartu 51003, tel. 7319800,
e-mail: peeter.padrik@kliinikum.ee
Praegune töökoht: direktor, hematoloogia-onkoloogia kliinik, Tartu Ülikooli Kliinikum

Haridus

Hugo Treffneri Gümnaasium, 1984
Tartu Ülikool, Arstiteaduskond, ravi eriala, 1990
Internatuur kirurgia erialal Tartu Kliinilises Haiglas, 1990–1991

Teenistuskäik:

Tartu Ülikooli Kliinikum, hematoloogia-onkoloogia kliinik, direktor, alates 2000
Tartu Ülikooli Kliinikumi Onkoloogiahaigla, peaarst 1997–1999
Tartu Ülikooli Kliinikumi Onkoloogiahaigla, peaarsti asetäitja 1996–1997
Tartu Ülikooli Kliinikumi Onkoloogiahaigla, onkoloog 1993–1996
Eesti Onkoloogiakeskus, onkoloog 1992–1993
Eksperimentaalse ja Kliinilise Meditsiini Instituut, nooremteadur, 1991–1992

Erialane enesetäiendus

Euroopa Onkoloogiakooli kursused:
- Rinnavähi kirurgiline ravi, 2000
- Kliinilised uuringud onkoloogias, 1996
- Molekulaarbioloogia onkoloogias, 1996
- Kolorektaalne kartsinoom, 1995
- Maksa ja kõhunäärme kartsinoom, 1994
- Rekonstruktiivne kirurgia onkoloogias, 1994
- Rinnavähk, 1993

EORTC kursus „Vähktõve Kliinilised Uuringud“, 1996
Štažeerimine Göttingeni Ülikooli Kliinikumis, 1996
Helsingi Kirurgiakliinikus, 1995
Odense Ülikooli Kliinikus, 1992
Taani Tervishoiuinstituudi kursus “Health care management”, 1999
Rahvusvahelised Euroopa ja Ameerika Vähikongressid ja kursused, 1995–2007

Teaduslik ja arendustegevus

Peamised uurimisvaldkonnad: neeruvähi, rinnavähi ja jämesoolevähi ravi. Vastutav uurija 16-s kliinilises uuringus.
Teaduslikud publikatsioonid: 4 artiklit rahvusvahelistes eelretsenseeritavates ajakirjades, 3 artiklit ajakirjas Eesti Arst, 8 teesid rahvusvahelistel konverentsidel.

Kuuluvus teadusseltsidesse:
Eesti Onkoloogide Selts, juhatuse liige
European Society of Medical Oncology, liige
American Society of Clinical Oncology, liige
Eesti Onkoteraapia Ühing, juhatuse esimees

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